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Gaseous Nitrogen Emissions from Caged Laying Hen Manure.

Emma Victoria Pratt

BSc (Hons)

A thesis submitted in partial fulfilment of the requirements of the
Open University for a degree of Doctor of Philosophy

20th May 2005

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DATE OF SUBMISSION 20 MAY 2005
DATE OF AWARD 23 MARCH 2006

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Abstract

A series of replicated, randomised block experiments were conducted to quantify the effect of temperature and egesta moisture content on the nitrogen losses during caged laying hen manure storage and to determine if the addition of a carbonaceous substrate to fresh caged laying hen egesta would reduce these losses.

During short-term storage (10-days), increasing temperature (15.3 - 25.4°C) and initial moisture content (722 – 796 g/kg) produced a linear increase ($P<0.01$) and a non-linear increase ($P<0.001$) in nitrogen loss from the manure, respectively. The interaction between these variables ($P<0.05$) produced a large increase in the rate of nitrogen loss from intermediate moisture samples, stored at the highest examined temperature. During long-term (18-weeks) manure storage, increasing temperatures produced a non-linear increase ($P<0.05$) in the rate of nitrogen loss

The addition of carbohydrates (initially at 8 g/kg, for 7-days) to laying hen manure was examined to reduce nitrogenous losses during storage. This showed that straw had no effect on the loss of nitrogen; glucose additions increased the loss of nitrogen and starch additions tended ($P>0.05$) to increase bacterial numbers. The addition of sucrose or maltose to the manure increased bacterial numbers ($P<0.05$) and gaseous nitrogen losses were reduced ($P<0.05$). Addition of increasing levels of sucrose (0-50g/kg) gave a non-linear reduction in nitrogen loss ($P<0.01$). The lowest level of nitrogen loss was obtained with 35 g/kg of added sucrose.

The application of sucrose at this inclusion level and the storage of the amended manure over 12 weeks showed that nitrogen losses were reduced for up to the fourth week of storage and thereafter there was no difference in nitrogenous losses.

Declaration

I hereby declare that this thesis has been composed by myself and has not previously been submitted for any other degree or qualification, to this or any other university. The work described herein is my own and all work of other authors is duly acknowledged.

Emma Victoria Pratt.

BSc (Hons)

Publications arising from this thesis

PRATT, E.V., ROSE, S.P. & KEELING, A.A. (2001) Effect of ambient temperature on nitrogen losses from stored laying hen manure. *British Poultry Science*, **42**: S73-74.

PRATT, E.V., ROSE, S.P., KEELING, A.A. & KERBAUL, E. (2002) Reducing nitrogen loss from laying hen manure by the addition of carbon-based amendments during short-term storage. *British Poultry Science*, **43**: S63-64.

PRATT, E.V., ROSE, S.P. & KEELING, A.A. (2002) Effect of ambient temperature on losses of volatile nitrogen compounds from stored laying hen manure. *Bioresource Technology*, **84**: 203-205.

PRATT, E.V., ROSE, S.P. & KEELING, A.A. (2004) Effect of moisture content and ambient temperature on the gaseous nitrogen loss from stored laying hen manure. *British Poultry Science*, **45**: 301-305.

PRATT, E.V., ROSE, S.P. & KEELING, A.A. (2005) Reducing gaseous nitrogen loss from stored laying hen manure by the addition of carbohydrates. *British Poultry Science In Press*.

Acknowledgements

I would like to thank my supervisors Dr Paul Rose and Dr Alan Keeling for their support throughout this project. I would also like to thank Jan Pemberton for her support, expertise and encouragement throughout this project.

Thanks are also due to all the people who helped collecting manure for the trial work and those who provided assistance with the laboratory analysis:

Steve Craddock, John Protheroe, Linda Kirby, Vassil Pirgozliev, Jan Pemberton, Tom Bridges, Paul Rose, Alan Keeling, Jackie Russell, Dave Handford, Clive George, Victoria Talbot, Andrew Bentham, Richard Page, Carol Walker, Chris Gee & Judith Wade.

Also I would like to thank all the library staff and computing technicians for all their help and assistance.

I am grateful to Labcell Ltd for the loan of the Decagon, Aqualab series 3, water activity meter; to the Oldacre foundation for my funding and to Dave Chadwick, Mick Worrall & Richard James for allowing me to collect manure from the commercial unit and their patience and consideration during these manure collections.

I acknowledge the assistance of Sciantec for providing the sodium analysis; the assistance of Erwan Kerbaul for his assistance in conducting experiment 8.1 and the assistance of Malcolm Pratt for creating the document template.

I would especially like to thank my family and friends, as without their un-wavering support and love, this project would never have been completed.

Finally, to Tom, for never once doubting.

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1. Introduction.

The U.K. edible egg production industry produces approximately one and a half million tonnes of manure each year (DEFRA, 2005). This quantity of manure would initially contain about 24,000 tonnes of total nitrogen (Phillips & Chambers, 2002). Manure from laying hens can be a valuable agricultural fertiliser, but the location of many layer enterprises and local agricultural nitrogen-use regulations may restrict the amount and timing of its application to the land. It is generally the responsibility of the egg producer to store the manure until it is required. Over half of the nitrogenous emissions occur during the housing and storage period (Phillips & Chambers, 2002).

The EC Integrated Pollution Prevention & Control Directive (Council Directive 1996/61/EC) designates large pig and poultry units (2,000 places for production pigs (over 30kg), or 750 sow places & 40,000 places for poultry) as regulated activities, which require a permit. As such, new installations and existing installations from 2007 must adhere to a code of practice and are required to reduce emissions of a range of pollutants, which includes ammonia. Installations that fall into this bracket must take measures to reduce emissions using the best available technology. This and other associated legislation aims to ensure industries cause a minimal amount of pollution, disposing of their wastes in an appropriate manner and use energy and raw materials efficiently. This will require them to consider their production process in its entirety and minimise wastes and ensure optimal use of input materials. The introduction of these new legislative requirements may provide the impetus for enterprises to introduce new methods of manure management in order to reduce ammonia emissions.

Once produced, laying hen manure is stored for varying lengths of time within the house and then may either be stored in a pit within the house or removed to an

alternative storage area. Nitrogen is lost from stored laying hen manure as ammonia which results from the microbial decomposition of the nitrogenous components of the manure (uric acid and un-digested proteins). Ammonia causes problems within the housing system with respect to the health and productivity of the stock (Charles & Payne, 1966a & b) and the health of the stock workers (Whyte, 1993). Ammonia losses to the environment are also undesirable as ammonia combines with naturally occurring and industrially-produced acidic species, which can acidify natural ecosystems (ApSimon *et al.*, 1987) and increase the nitrogen loading of nitrogen sensitive ecosystems (Sutton *et al.*, 1995). A number of studies have investigated the source of the ammonia from poultry units and considered how various environmental or production parameters affect its formation and release. However, most of these studies have utilised poultry litter not poultry manure. Poultry litter contains a source of carbonaceous bedding material such as woodshavings, sawdust or straw. Manure from caged laying hens is simply the egesta and excreta of the hen mixed with some waste feed and possibly some broken or shell-less eggs. Without the source of carbonaceous material, poultry manure decomposes differently to poultry litter mostly because of the differing carbon to nitrogen (C: N) ratio. Thus, models created to explain the effect of environmental variables on the ammonia loss from litter may not be appropriate for laying hen manure. There is a clear need to identify how aspects of the production process affect ammonia emission from laying hen manure, in order to recommend management techniques to reduce emissions.

Optimising the nitrogen content of the diet is a potential method of reducing nitrogen emissions from laying hen manure. The dietary requirements of laying hens have been well established and optimum levels have been defined for maximum productivity and efficiency. Though reductions in dietary nitrogen are possible, the reformulation of the

diets and amino acid supplementation will incur costs to the industry, in which effective diet formulation is dominated by the economics of production. It is not the focus of this thesis to examine this method of reduction further, but to examine more strategic methods of reducing nitrogen loss.

The poultry industry, governing bodies and planning authorities need to decide what types of housing systems they are going to recommend as being the most suitable for the production of edible eggs and how the manure is to be stored to maximise its value as an organic fertiliser and minimising pollution. The most potentially effective methods of controlling nitrogenous losses from laying hen housing systems are the control of ambient temperature and manure moisture levels. Laying hen housing systems mainly vary in the way in which manure is stored and the temperature of the manure store. Deep-pit systems store the manure within the housing system and most ventilate the warm exhaust air through the pit area, whilst belt-cleaned and slatted floor systems frequently remove the manure to a separate storage area. Reducing the temperature at which the manure is stored could substantially reduce the environmental nitrogen loss. There is a need for quantitative data that describes the effect of different manure storage temperatures on nitrogen loss.

Manure moisture content also has a profound effect on the rate of nitrogen loss from laying hen manure. The minimisation of manure moisture levels could easily be achieved by optimising dietary mineral levels (Smith *et al.*, 2000a), improving quality control at feed mills to prevent dietary excesses (Smith *et al.*, 2000b), ensuring good drinker design and management (Phillips & Chambers, 2002) and by the use of drying systems (Van Horne, 1997). However, these methods of control would result in additional costs to the producer. There is a need for quantitative data that describes the effect of different manure moisture contents on nitrogen loss.

The management of laying hens, in for example belt clean housing systems, results in the regular mechanical removal of newly-produced manure. This gives an easy opportunity to add materials to the manure to reduce ammonia loss. Some substrates such as acids, zeolites, *yucca* and formaldehyde have been tested in litter-based systems. The effects of these amendments are to restrict microbial activity so preventing ammonia production. The restriction of microbial activity has been suggested to potentially cause problems if the intended use of the manure is as an organic fertiliser, because the un-stabilised manure may result in nitrogen toxicity to the crops (Barberis & Nappi, 1996).

Nitrogen loss from laying hen manure is high because the manure has a low C: N ratio as there is no litter material present. Thus, an alternative to traditional manure management is the addition of a carbon source to the laying hen manure when it is removed from the caged area. This may increase microbial activity allowing the manure to be managed more like compost than manure. The C: N ratio of the manure is increased by the addition on a carbonaceous substrate, which is thought to enable a reduction of nitrogen losses by increasing the microbial population. Hence, the nitrogen is incorporated into the microbial biomass preventing its volatilisation (Atkinson *et al.*, 1996). Some reductions in nitrogen losses have been produced by increasing the C: N ratio of animal manures and slurries by the addition of carbohydrates (Witter & Lopez-Real, 1987). Similarly again, this reduction in ammonia emission probably occurs as a result of nitrogen being incorporated within the microbial biomass (Atkinson *et al.*, 1996). The addition of an available carbon source is a novel approach to reducing nitrogen emissions from fresh laying hen manure. There is a need to investigate the

possibility of enhancing the microbial decomposition process to prevent ammonia emission, through the addition of carbonaceous amendments.

The specific objectives of this thesis were:

- To quantify the effects of ambient temperature and manure moisture content on the ammonia loss and their interactions during laying hen manure storage.
- To investigate the amendment of fresh laying hen manure with carbonaceous substrates of increasing resistance to microbial hydrolysis to reduce nitrogen losses during short-term storage.
- To establish an optimum inclusion rate for the carbonaceous manure amendment and to determine the effects of the carbon amendment on laying hen manure during long-term storage.

2. Ammonia.

Ammonia is the most common basic gas in the atmosphere (Harrison, 1990); it is colourless, highly soluble in water and has irritant qualities (Jarvis & Pain, 1990; Carlile, 1984). There are no known chemical processes that generate ammonia directly in the atmosphere; therefore all ammonia has a biological origin (Wayne, 1991).

Ammonia has a residence time of only a few days in the atmosphere and as a consequence is not well mixed in the atmosphere (Jenkinson, 1990). There is an exponential decrease in aerial concentrations with increasing distance from emission sources, as a result of its reactivity with other compounds (Fowler *et al.*, 1996) (Section 2.1). The annual budget is many times more than the amount of ammonia in the air at any one time, as it is mostly deposited close to its source or converted into aerosols (Jenkinson, 1990). Ammonia, as aerosols may reach high levels in the atmosphere and travel long distances before being deposited in rainfall, known as “wet deposition” (ApSimon & Kruse-Plass, 1991; Sutton & Fowler, 2002). “Dry deposition” also occurs, when gases and particles are absorbed by land, vegetation or water surfaces (Van Breemen *et al.*, 1982; Sutton & Fowler, 2002). Dry deposition accounts for the major pathway of ammonia removal from the atmosphere, whilst wet deposition accounts for the removal of the majority of the ammonium particles (Sutton & Fowler, 2002). The relative proportion of dry and wet deposition is largely related to the level of rainfall in the area.

Ammonia and its aerosols cause environmental damage when they are re-deposited to land or water, by the enrichment and acidification of natural habitats. Ammonia has been implicated in forest decline, plant species change and eutrophication of surface waters (Burton, 1997; Sharpe & Harper, 1995; Innes, 1993; Harrison, 1992; Wellburn,

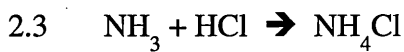
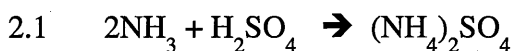
1988). The addition of excess nitrogen to sensitive ecosystems e.g. heathland or bogs, which exist partly due to naturally low soil nitrogen conditions is a typical example (MAFF, 1997; Sutton *et al.*, 1995; Roelofs & Houdijx, 1991). The U.K. is committed to the protection of such areas through the EC Habitats Directive (Council Directive 1994/43/EC). These areas may experience changes in the dominant species as plants with high nitrogen requirements are able to out-compete the areas' natural species (Hornung *et al.*, 2002), such as the transition of heathland to grassland (Roelofs & Houdijx, 1991). Although low levels of nitrogen enhance growth, excessive nitrogen results in changes in the nitrogen content of foliage, potentially increasing the plant's susceptibility to environmental stresses such as drought, frost and insect damage (Hornung *et al.*, 2002; WHO, 1987). Ammonia and its chemical combinations (NH_x) (described in section 2.1), are important components responsible for the acidification of the environment, by acidic deposition (ApSimon *et al.*, 1987; Van Breemen *et al.*, 1982; Möller & Schieferdecker, 1985; Burton, 1997; MAFF, 1997; Harrison *et al.*, 1991). The acidification of natural habitats again causes shifts in dominant plant species, with the loss of acid intolerant species (Roelofs & Houdijx, 1991; Hornung *et al.*, 2002). The bacterial conversion of the ammonium species to nitrate in the ground leads to nitrate leaching and subsequent enrichment of surface and ground waters (Hornung *et al.*, 2002). Ammonia can have direct toxic effects upon vegetation (Colls, 2002; Burton, 1997). Vegetation growing close to a strong emitting source may exhibit damaged foliage and slower growth (Roelofs & Houdijx, 1991; MAFF, 1997).

Legislation that is designed to reduce acid rain and protect sensitive habitats has been developed within the European Community and has been adopted by the UK. The Protocol to Abate Acidification, Eutrophication and Ground-Level Ozone (Gothenburg Protocol) of the UNECE Convention on Long-Range Transboundary Air Pollution

(adopted in 1999) and the EC National Emissions Ceiling Directive (Council Directive 2001/81/EC) require the UK to reduce ammonia emissions to 297 kilo tonnes (kt) per year by 2010.

2.1 Ammonia pollution chemistry

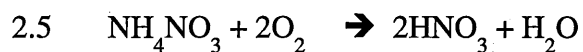
Ammonia combines easily with acidic species present in the atmosphere (Equations 2.1, 2.2 and 2.3) forming neutral ammonium aerosols (Sharpe & Harper, 1995; Innes, 1993; ApSimon & Kruse-Plass, 1991; Wayne, 1991; Harrison *et al.*, 1991; Harrison, 1990; ApSimon *et al.*, 1987; Wood & Holliday, 1960).



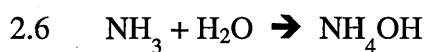
Source: - Sharpe & Harper, 1995; Harrison *et al.*, 1991; Wayne, 1991

Ammonia can be transported over long distances in the form of ammonium aerosols (ApSimon & Kruse-Plass, 1991). In areas with large sources of ammonia, for example agricultural areas, the oxidation and deposition of acid gases are likely to be greatly increased (Kruse, 1986). Although ammonia is not acidic, the ammonium aerosols that are formed in the atmosphere (equations 2.1, 2.2 and 2.3) are rapidly oxidised on their return to earth. This occurs under the influence of soil bacteria, which are instrumental in the release of the parent acids (equations 2.4 and 2.5) (MAFF, 1997; Innes, 1993; Wayne, 1991; Harrison, 1990; Klarenbeek & Bruins, 1988; ApSimon *et al.*, 1987).





Under normal temperature and pressure conditions, ammonia is present as a gas with a high affinity for water (Kruse, 1986). It is highly soluble at low and moderate pH; it is often dissolved in water (equation 2.6) and returned to earth via precipitation contaminating the local area (Wayne, 1991; Shuler, 1980). Hartung (1988) found that the quantity of ammonium ions present in rainwater collected in agricultural areas was considerably higher than in non-agricultural areas, which confirms the local nature of the ammonia emissions.



3. Sources and Production of Ammonia.

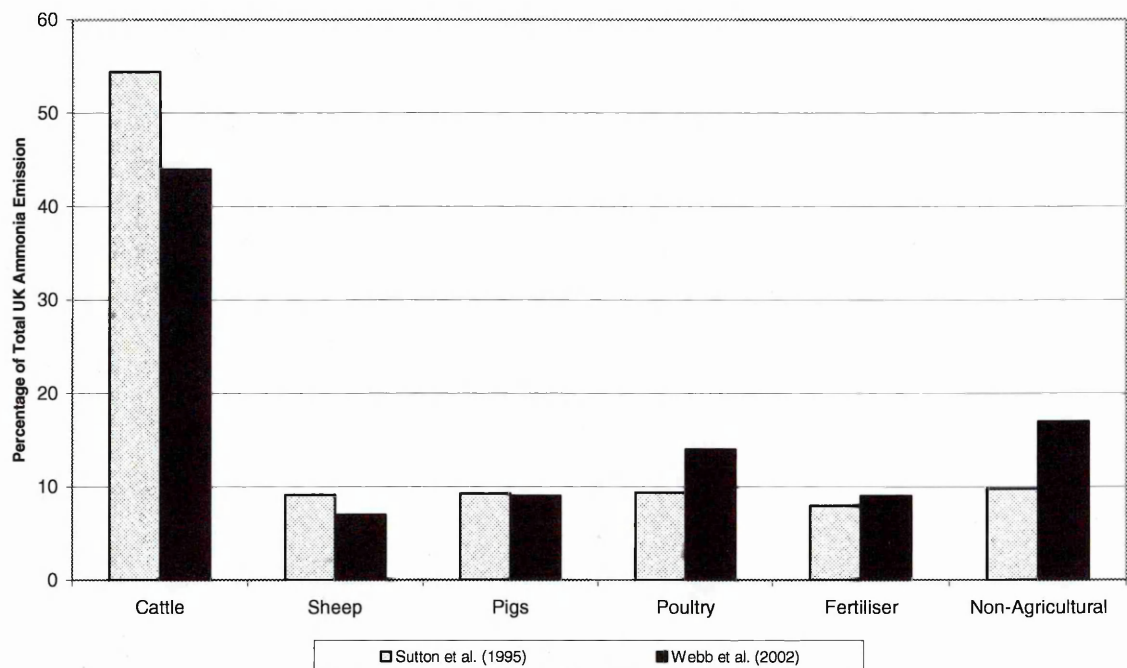
Over half of the global ammonia emissions originate from livestock operations (Table 3.1, Colls, 2002). However, providing quantitative estimates of these emissions is difficult due to the wide range and variability of factors that affect the emission rate (Fowler *et al.*, 1996). In the UK, 85-90% of ammonia is produced from agricultural sources (Webb *et al.*, 2002b; Sutton *et al.*, 1995). Ammonia is produced by the decomposition of the nitrogenous components in the manure, urea in animal manure or uric acid in poultry manure (Webb *et al.*, 2002a; Groot Koerkamp, 1998; Fowler *et al.*, 1996; Sutton *et al.*, 1996; Williams, 1995).

Table 3.1 Global ammonia emissions (Colls, 2002).

Source	Global Emission
	Mega tonnes of Nitrogen / Annum
Vegetation	5
Oceans	7
Biomass Burning	2
Fertiliser Application	6
Livestock	25
Total	45

Ammonia emission estimates made by Sutton *et al.* (1995), that are consistent with previous work by Kruse *et al.* (1989) and Jarvis & Pain (1990), show cattle are the major source of UK emissions, producing over 50% of the ammonia. Sheep, pigs, poultry and fertiliser / crop emissions each estimated to contribute 8-9% towards the total. However, a more recent estimate of these emissions (Webb *et al.*, 2002b) shows a more significant contribution from the poultry industry, at 14% of the total UK emissions (Figure 3.1).

Figure 3.1 Source of ammonia emissions in the UK.



There has been an expansion of the poultry industry, both in terms of bird numbers and production sites which are now situated in close proximity to slaughterhouses, to reduce transport costs (Haque & Vandepopuliere, 1994). Moreover, this intensification has changed the pattern of ammonia emission from area sources to point sources (Colls, 2002). This will result in more specific areas of damage from ammonia pollution, close to units of production.

3.1 Production and composition of poultry manure.

The term poultry manure will be used to describe the collected fresh droppings (excreta) from systems that do not involve litter e.g. cage systems. In systems that involve a form of bedding, the term poultry litter will be used to describe the mixture of bedding and droppings.

In the U.K., 28 million laying hens are housed in caged systems (Phillips & Chambers, 2002). They produce in excess of 1.5 million tonnes of manure each year, typically

containing between 24,000 and 35,000 tonnes of nitrogen (Phillips & Chambers, 2002; Chambers & Smith, 1998). The quantity and composition of laying hen manure varies according to nutritional factors such as feed composition, intake and conversion, as well as age, breed, stage of production and water intake.

The nutrient content of poultry manure varies widely (Nicholson *et al.*, 1996), in general layer manure has a dry matter content of approximately 350 g/kg and a Nitrogen: Phosphorus: Potassium (N:P:K) ratio of 6:2:3 (Nicholson *et al.*, 1996). The readily available nitrogenous portion of the laying hen manure (ammonium and uric acid) accounts for approximately 50% of the total nitrogen.

3.2 Ammonia Production & Release from Laying Hen Manure.

Ammonia is produced from the microbial decomposition of uric acid, un-digested proteins and other nitrogenous compounds in poultry manure (Burnett & Dondero, 1969; Schefferle, 1965a, 1965b). This process commences almost immediately upon excretion of the manure (Chang & Flint, 1976).

The simplified biochemical processes are described by:



The non-biological decomposition of organic nitrogenous materials is kinetically very slow (Groot Koerkamp, 1994). Hence, both of these processes are microbially mediated (Kitai & Arakawa, 1979; Groot Koerkamp, 1994; Carlile, 1984; Burnett & Dondero, 1969; Schefferle, 1965b; Bacharach, 1957). Evidence of both aerobic and anaerobic activity has been discovered (Section 3.4).

The protein portion of the waste (approximately 26% crude protein and 11% true protein, Shuler, 1980) occurs as a result of un-digested feed protein present in the faeces. While non-protein nitrogen is present as uric acid (approximately 6.5%, Shuler, 1980) which is the excreted portions of amino acid metabolism.

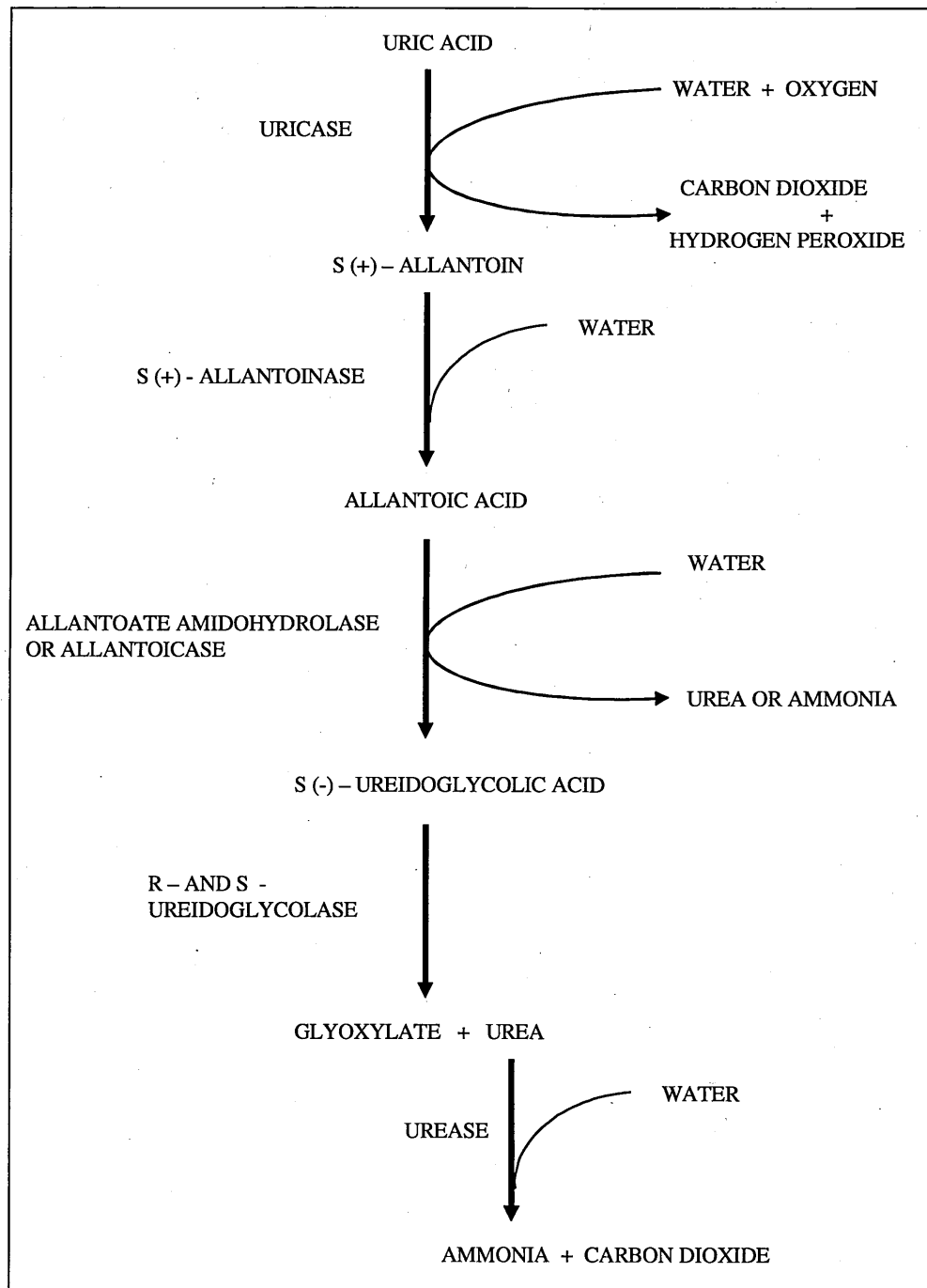
The decomposition of uric acid is a two step process with urea formed as an intermediate product (Elliott & Collins 1982; Schefferle, 1965b), requiring the presence of water and oxygen (Carlile, 1984; Groot Koerkamp, 1994) (Section 3.3). Once the ammonia is formed it will be in one of two forms, as ammonia gas or as the ammonium ion, depending on the pH of the medium (Elliott & Collins, 1983). The amount of ammonia volatilised is influenced by a number of factors that directly affect the ammonia / ammonium equilibrium. This equilibrium is mainly affected by pH and is explained in more detail in Section 4.2.1. The degradation of uric acid and un-digested proteins in manure and litter is mainly influenced by dry matter, temperature, pH and surface to air contact (Groot Koerkamp, 1994; Von Wachenfelt, 1993; Kirchmann & Witter, 1989; Voorburg, 1986; Wellburn, 1988; Carlile, 1984) (Section 4).

3.3 Uric acid decomposition.

The decomposition of uric acid (Figure 3.2) to produce ammonia and carbon dioxide is the result of a series of reactions that requires oxygen and water. Urea is formed as an intermediate product (Vogels & Van Der Drift, 1976). The enzyme uricase is specific to this reaction and is commonly present in microorganisms (Groot Koerkamp, 1994). Whilst studying uricase, Baum *et al.* (1956) found that the peak rate of uric acid disappearance occurred at pH 9.0 and the rate of disappearance decreased with more acid or alkaline pH conditions. The microorganisms that convert uric acid to urea and ammonia thrive in alkaline conditions (Carlile, 1984). The bacteria that decompose uric

acid are predominantly aerobic (Schefferle, 1965b) and the majority of these organisms only decompose uric acid to the intermediate urea, though some do produce ammonia as an end product.

Figure 3.2 The simplified aerobic decomposition of uric acid (Vogels & Van Der Drift, 1976).



Burnett & Dondero (1969) examined the rate of loss of uric acid from “dry” (250 g/kg dry matter) and “liquid” (20 g/kg dry matter) poultry manure. They found over the first 7 days that 89% and 99%, respectively, of the initial uric acid content was lost from dry and liquid manure. This disappearance of uric acid was shown to coincide with the increased production and release of ammonia (Burnett & Dondero, 1969; Figure 3.3). Though these workers did not perform microbial analysis on the “dry” poultry manure, aerobic and anaerobic uric acid decomposing bacteria were isolated from the “liquid” manure (1×10^{10} & 1×10^8 cfu per g dry weight of manure, respectively). The high level of anaerobic activity would have occurred as a result of the high moisture content, which would have provided poor aerobic conditions. However, due to the disturbance created by sampling, aerobic activity was also present. The anaerobic activity detected by Schefferle (1965b) in poultry litter represented only 0.0002% of the total uric acid decomposing bacteria isolated during her investigation. This may have been a result of the litter substrate providing a structure to the waste, which maintained good aerobic conditions. When cold, wet poultry litter was tested, a decrease in aerobic bacteria was detected (Schefferle, 1965a) and this indicates that the structure of the litter is compromised when it becomes wet.

A number of factors affect the metabolism of the heterogeneous microbial population and hence uric acid breakdown rates (Elliott & Collins, 1982). In particular, the wide-ranging temperatures, litter pH and moisture contents encountered in poultry houses have a substantial effect on microbial activity (Figure 3.4 and Figure 3.5). As the temperature rises in the manure, the decomposition rates increase with a rapid increase between 20 and 30°C (Groot Koerkamp, 1994). At pH 5.5 and above, increased breakdown rates occur, with an optimum of approximately pH 9 for uricase (Baum *et al.*, 1956). Microbial growth in litter is optimal between 40 and 60% moisture content

(Elliott & Collins, 1983; Figure 3.5). At values above and below this range, ammonia release decreases. Theoretically the ammonia release stops completely at very low moisture contents. As poultry litters generally have moisture contents around 40%, an increase in moisture content normally produces an increase in ammonia release (Groot Koerkamp, 1994). However, Schefferle (1965a) found no correlation between these factors and microbial populations, and hence ammonia.

Figure 3.3 Uric acid decomposition and ammonia evolution from dry (250 g/kg dry matter) poultry manure (Burnett & Dondero, 1969).

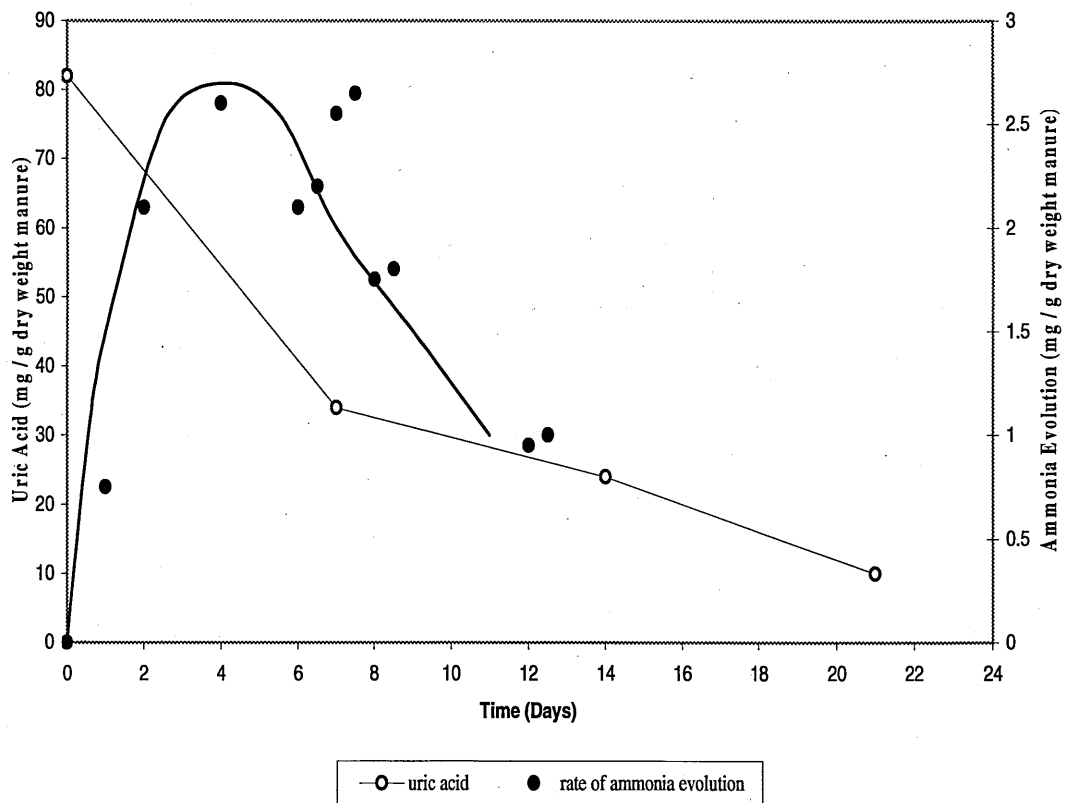
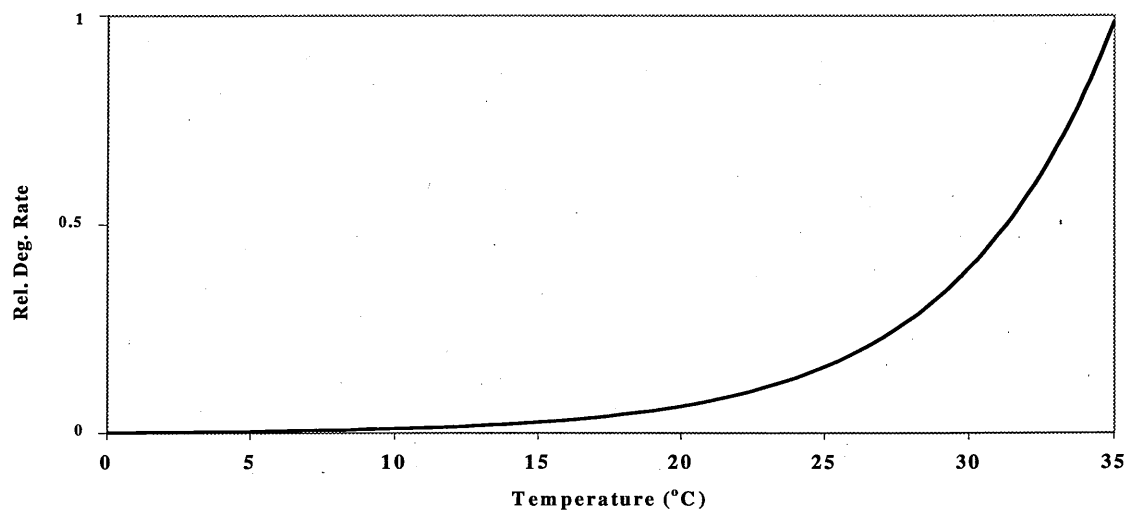
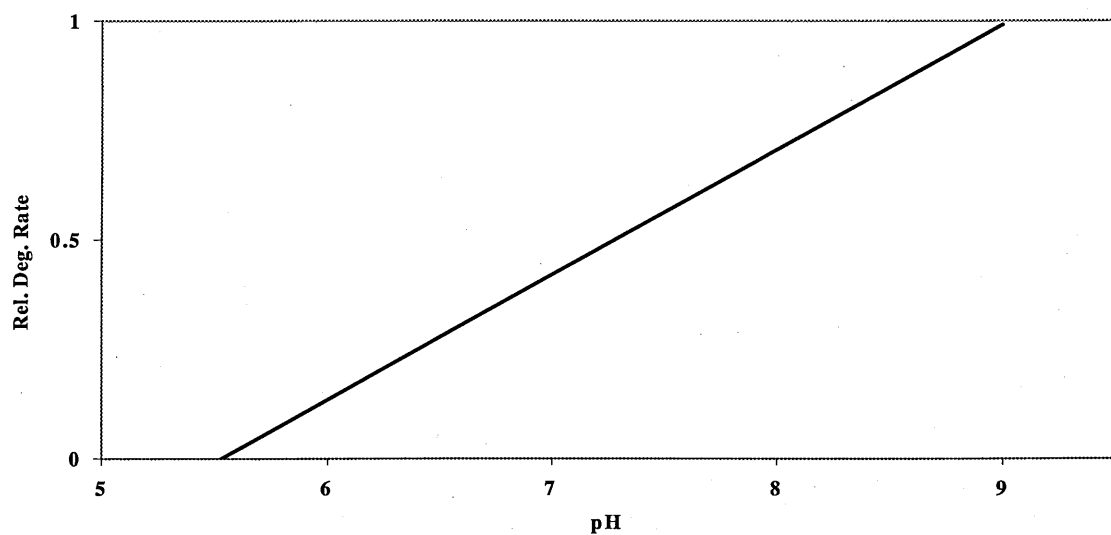


Figure 3.4 The effect of temperature (A), pH (B) and water activity (C) on the relative degradation rate of uric acid (Groot Koerkamp, 1994).

A



B



C

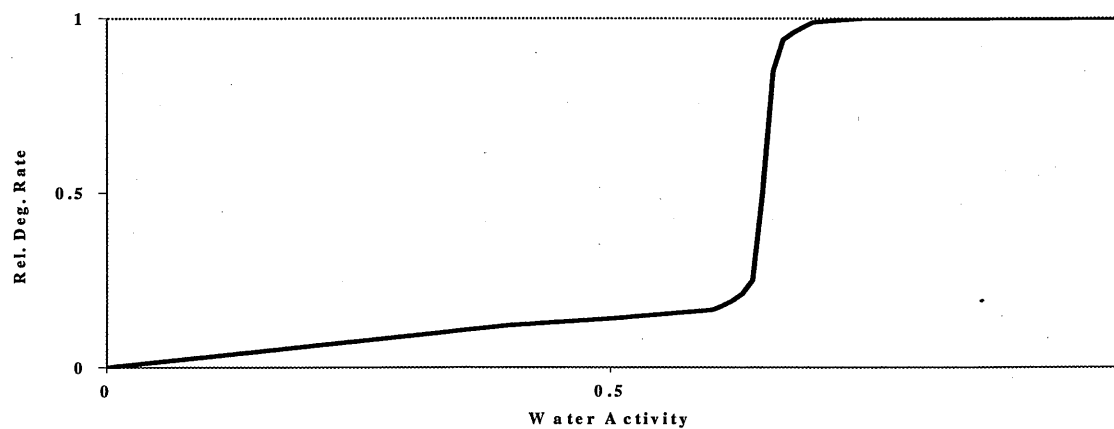
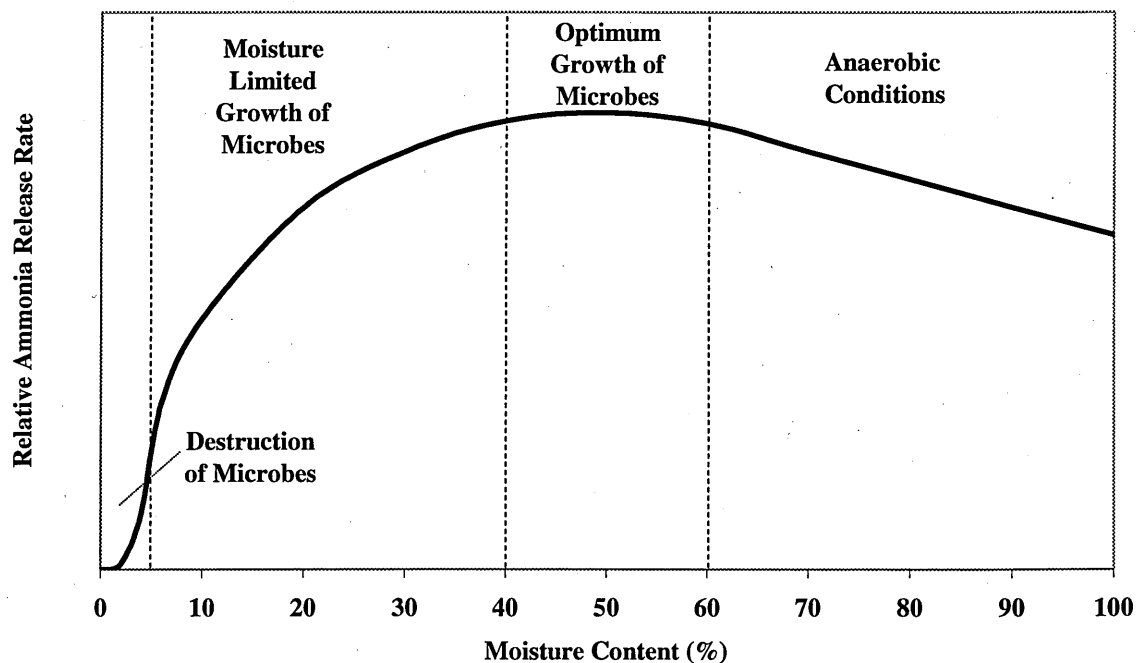


Figure 3.5 The effect of litter moisture content on the microbial release rate of ammonia (Elliott & Collins, 1983).



3.3.1 Urea decomposition.

The decomposition of urea is the last step in the decomposition of uric acid to ammonium / ammonia (Figure 3.2). The hydrolysis of urea occurs rapidly in the presence of urease, which is commonly present in manure (Groot Koerkamp, 1994). Studies of the rate of urea decomposition (Varel *et al.*, 1997; Varel, 1996; Elzing & Monteny, 1997a & b) agree that urea decomposition is normally complete within 24 hours, with the majority of the urea disappearing within the first 2-3 hours. Varel (1996) demonstrated the corresponding disappearance of urea and appearance of ammonia in slurry and the subsequent volatilisation of ammonia to the atmosphere, which followed the same exponential pattern of ammonia evolution and urea decomposition as shown for uric acid by Burnett & Dondero (1969 - Figure 3.3).

The decomposition of urea is also reduced at low temperature and pH (2% of urea lost at pH 4.6 and 20% of urea lost at pH 8.5; Wahab *et al.*, 1960). Attempts to prevent ammonia formation in the gut of poultry were successful by feeding a urease inhibitor, *Yucca schidigera* (Balog *et al.*, 1994). They found gut ammonia levels were reduced, but supplementation of the diet with increasing levels of *yucca* extract produced a dose response increase in atmospheric ammonia concentrations (Anthony *et al.*, 1994). The addition of urease inhibitors to slurry was shown to be effective but only for a limited period of up to 2 weeks (Varel, 1996). As a result, it can be concluded that such a technique would only be effective with repeated application of the urease inhibitor.

3.4 Microbiology of litter and manure.

The microbial population of a substrate is dependent not only on the initial inoculum but also on the environment in which it is situated. The growth of microbial populations often follows a similar pattern (Figure 3.6). Jackson *et al.* (1970), showed that the lag phase lasted only 24 hours for microorganisms grown in poultry manure, observed this pattern of growth.

Kitai & Arakawa (1979) illustrated the role of microorganisms in ammonia release by sterilising broiler chicken excreta (121°C for 20 minutes). Incubation of the sterilised material (at 33°C for 24 hours) yielded little ammonia gas. The study of the microbial contents of poultry litter and poultry manure has found a large range in numbers of aerobic and anaerobic microorganisms present, the findings of some of these studies are summarised in Table 3.2. Aerobic bacterial numbers are usually 1×10^8 and 10^{10} with higher levels present in litter than manure (Giddens & Rao, 1975; Schefferle, 1965a; Halbrook *et al.*, 1951). Anaerobic bacteria are generally present in lower numbers (1×10^4 and 10^8), though this lower level may be a result of enumeration techniques which

may lose viability in these organisms. The bacteria grown from stored poultry litter could be separated into three main groups: coryneform bacteria, micrococci and gram negative type bacteria (Schefferle, 1965a). Although there is little variation in the total counts, the proportion of the different types varies with the alkalinity of the samples. Generally an increase in alkalinity shifts a significant proportion of the total population to coryneform bacteria from the gram-negative types (Schefferle, 1965a).

Figure 3.6 The generalised growth curve of a microbial population, based on Freeland (1991).

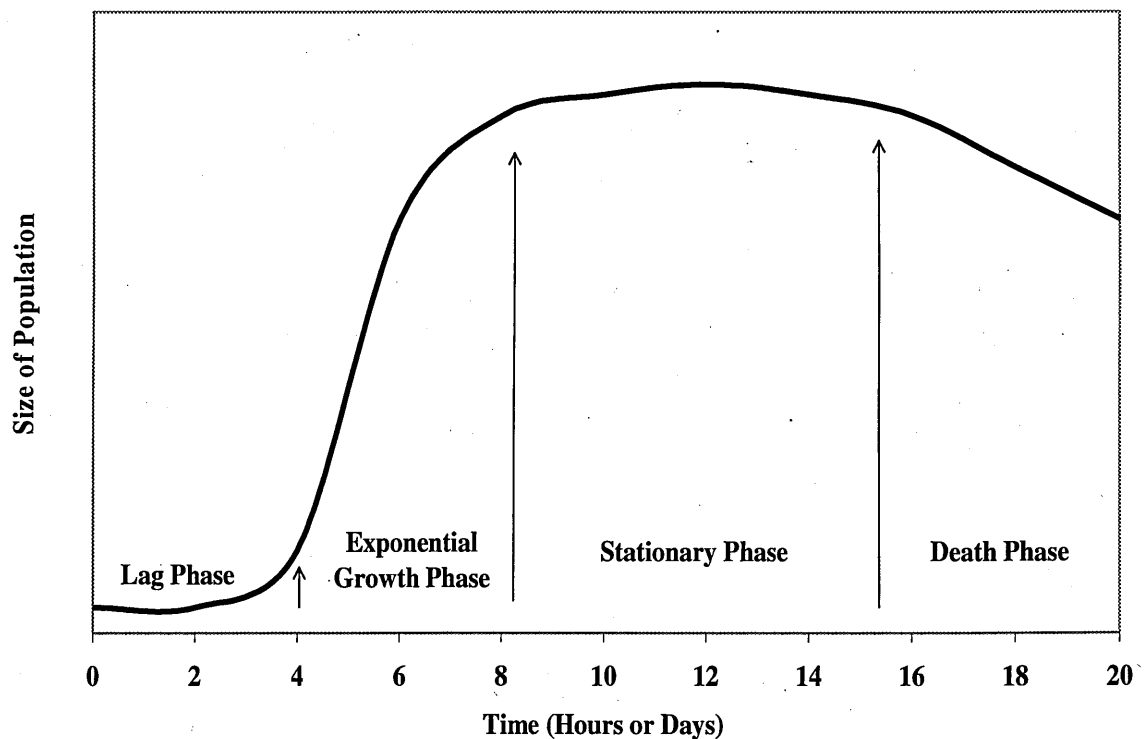


Table 3.2 The number of microorganisms detected in poultry manure and poultry litter using aerobic and anaerobic enumeration techniques.

Number of Microorganisms Detected			Reference
	Poultry Manure	Poultry Litter	
Aerobic	8.0 x 10 ⁷ – 1.6 x 10 ⁹ / ml of culture		Jackson <i>et al.</i> (1970)
	8.0 x 10 ⁷ / g (Fresh Weight)	1 x 10 ¹⁰⁻¹¹ / g (Fresh Weight)	Schefferle (1965a)
		2.2 – 64 x 10 ⁹ / g (Fresh Weight) (1)	Schefferle (1965b)
	1 x 10 ¹⁰ / g (Dry Weight)		Burnett & Dondero (1969)
		1 x 10 ⁸ – 2 x 10 ¹¹ / g (Fresh Weight)	Lovett <i>et al.</i> (1971)
	1 x 10 ⁹ / g	1 x 10 ⁹ / g	Halbrook <i>et al.</i> (1951)
		1 x 10 ⁶ – 3 x 10 ⁸	Ivos <i>et al.</i> (1966)
	1 x 10 ⁶ CFU (2)		Nodar <i>et al.</i> (1990a)
		1.1 x 10 ⁶	Nodar <i>et al.</i> (1990b)
		1 x 10 ⁷ / g	Johnson <i>et al.</i> (1985)
	6 x 10 ³ – 3 x 10 ⁶ (3)		Chang <i>et al.</i> (1974)
	9 x 10 ⁹ / g	8 x 10 ¹⁰ / g	Giddens & Rao (1975)
Anaerobic		8 x 10 ¹⁰ – 14 x 10 ¹⁰	Atkinson <i>et al.</i> (1996)
	7.0 x 10 ⁵ – 1.1 x 10 ⁸ / ml of culture		Jackson <i>et al.</i> (1970)
		<1 x 10 ⁴ / g (Fresh Weight) (1)	Schefferle (1965b)
	1 x 10 ⁸ / g (Dry Weight)		Burnett & Dondero (1969)
		2 x 10 ⁷ – 2 x 10 ¹⁰ / g	Lovett <i>et al.</i> (1971)
	1 x 10 ⁸ / g	1 x 10 ⁸ / g	Halbrook <i>et al.</i> (1951)
	1 x 10 ⁴ CFU (2)		Nodar <i>et al.</i> (1990a)

(1) Organisms Only Decomposing Uric Acid.

(2) CFU = Colony Forming Units.

(3) Manure dehydrated prior to culture.

A range of factors (temperature, moisture content, pH and oxygen availability) can alter microbial growth (Jay, 1996), as well as variables such as nutrient availability and the ratio of minerals present (C:N).

3.4.1 Fungi, Moulds and Yeast.

Bacon & Burdick (1977) found 18 species of fungi capable of growing in broiler litter during the crop cycle. Also, Lovett *et al.* (1971) isolated 17 species from litter. Schefferle (1965a) found a slight decrease in the amount of moulds and yeast with increased length of storage of the litter (Chopped straw and peat moss litter) (Table 3.3) and also noted that these counts were reduced by strong alkaline conditions.

Table 3.3 Plate counts of moulds and yeast in poultry litter and droppings (Schefferle, 1965a).

Sample Type	Age of Litter	pH of sample	Plate count ($1 \times 10^3 \text{ g}^{-1}$ fresh weight)	
			Moulds	Yeast
Chopped straw & peat moss	1 Week	7.2	11 000	88 000
	5 Weeks	7.9	20 000	3 000
	9 Weeks	8.0	5 400	1 000
	3 Months	7.6	3 100	200
	4.5 Months	7.6	920	90
	9 Months	6.4	1 300	1 000
Chopped straw	10 Weeks	7.4	4 200	<100
Chaff, peat, moss and straw	7 Months	8.1	70	180
Sawdust and cow manure	9 Months	8.5	100	20
Droppings	Fresh*	-	130	1 050

3.4.2 Microbial growth and Temperature.

Temperature variations cause the greatest effect of all the physical variables to which microorganisms are subjected (Porter, 1946). It can stimulate growth, alter metabolism or destroy the microorganisms depending on the intensity and period of exposure (Jay, 1996). Microorganisms thrive over a range of temperatures, with each species having a particular range, with the optimum temperature being closer to the maximum than the minimum (Freeland, 1991). Using the optimum temperature as a classification tool, microorganisms can be categorised into three distinct groups;

1. Psychrophiles ($<20^{\circ}\text{C}$),
2. Mesophiles ($20\text{--}45^{\circ}\text{C}$)
3. Thermophiles ($>40^{\circ}\text{C}$).

During manure storage and decomposition, temperatures are most likely to be above 20°C and may be as high as 70°C as a result of microbial self-heating (Dewes, 1999). As the temperature increase that is produced by microbial activity is likely to occur gradually, the progression of microbial growth from mesophilic organisms to thermophilic organisms also occurs gradually. Detrimental effects of temperature increase are unlikely to hamper decomposition. The effects of both environmental temperature and temperatures that are induced by microbial self-heating within the manure / litter substrate are discussed in section 4.3.

3.4.3 Microbial growth and Moisture Content.

Water is necessary for the growth of microorganisms (Groot Koerkamp, 1994). The water activity (A_w) of a substrate is a measure of the availability of water for microorganisms (Griffin, 1981). Water activity has been generally established as an

indicator of bacterial contamination in poultry litter (Carr *et al.*, 1993). Water activity may therefore be considered to be a better explanatory variable for microbial growth than moisture content (Groot Koerkamp, 1994), though Miller (1989) considered the use of water activity to be ineffective and insensitive when examining microbial growth in moist systems. Higher dry matter contents generally reduce water activity, but this is not the case for all materials (Groot Koerkamp, 1994). However, an A_w of 0.7 is generally considered to be the absolute minimum for microbial growth (Griffin, 1981). Gervais *et al.* (1988) and Grajek & Gervais (1987) showed that reduced water activity reduced bacterial growth rates.

Excessive moisture can also hinder aerobic microbial growth due to the onset of anaerobic conditions (Stentiford, 1996; Miller, 1989). This aspect is discussed in section 3.4.5. The effect of moisture content on ammonia loss from manure is discussed in section 4.4.

3.4.4 Microbial Growth and pH.

Different species of microorganisms grow at specific, optimum pHs (Porter, 1946). Most bacteria grow within the range of 6-8 (Lowrie & Wells, 1994) while molds, yeasts and fungi prefer slightly acid conditions i.e. pH 5-6 (Porter, 1946; Lowrie & Wells, 1994). As microorganisms metabolise and excrete waste products, the pH of the substrate can alter, changing the dominance of particular species within the substrate. The effect of pH on ammonia loss is discussed in section 4.2.

3.4.5 Microbial Growth and Oxygen Availability.

Microorganisms can be classified according to their oxygen requirements:

- Strict Anaerobes are organisms that are killed by just a slight amount of oxygen.

- Obligate Anaerobes are organisms that require an oxygen free atmosphere for growth.
- Facultative Anaerobes are organisms that grow aerobically when oxygen is available, whilst having less efficient mechanisms to allow function to continue in the absence of oxygen.
- Obligate Aerobes are organisms that require molecular oxygen for their metabolism.

(Lowrie & Wells, 1994)

The determination of aerobic and anaerobic populations discussed in section 3.4 does not allow for the range of classifications described here. Therefore it is likely that these populations have both been overestimated, as facultative anaerobes could be present in both aerobic and anaerobic counts. The effect of oxygen availability during the decomposition of manure is discussed in section 3.6.

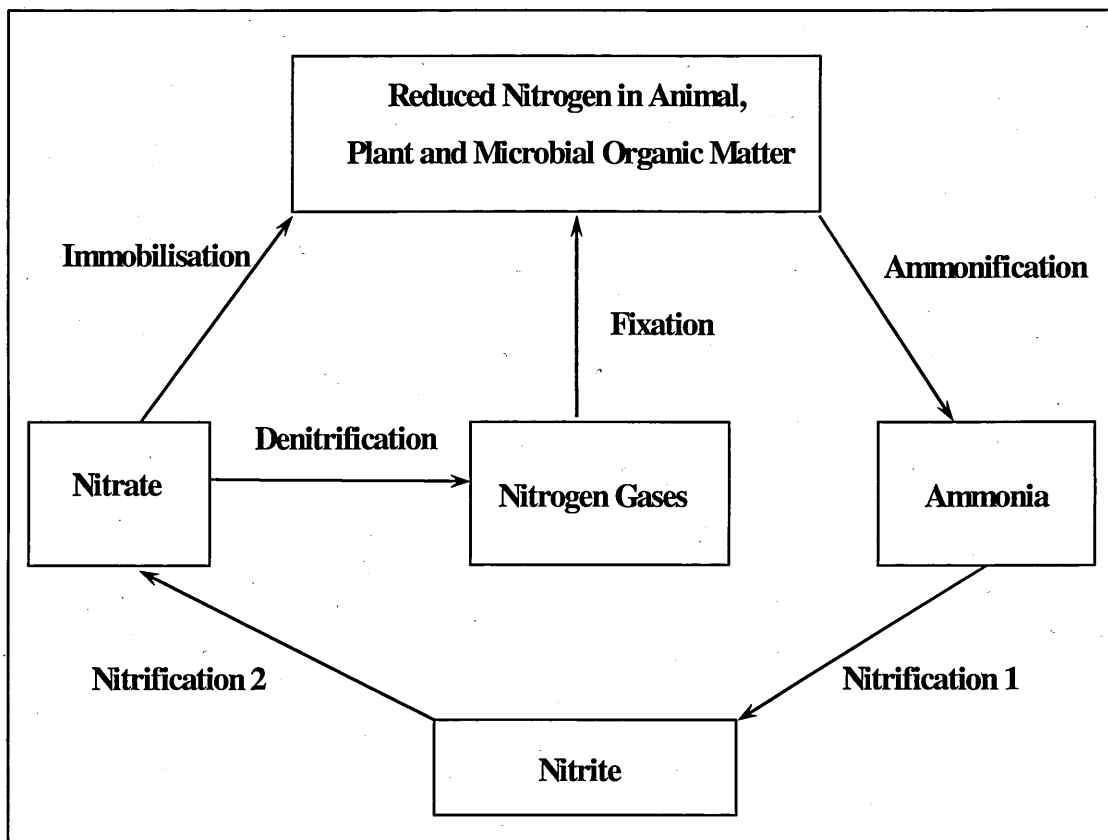
3.4.6 Microbial Growth and Nutrient Ratios.

Nitrogen and carbon are the major food sources required by microorganisms (Gray *et al.*, 1971). Microbial cells typically contain 50% carbon and 5% nitrogen (on a dry weight basis), requiring available nitrogen to be in the region of 2-4 parts per 100 of initial carbon e.g. a C: N ratio between 25:1 and 50:1 (Alexander, 1977). A wide range of carbon and nitrogen sources are degraded by microbes to allow cellular growth and division to occur. Carbon that is surplus to the direct requirements of the microbial population is released as carbon dioxide and surplus nitrogen is released as ammonia (Higgins & Burns, 1975). Only 20-40% of the initial carbon present is thought to be assimilated into microbial cells (Alexander, 1977). A high loss of carbon occurs from substrates that have a high initial C: N ratio, typically exceeding 30:1. Conversely, a low C: N ratio (below 10:1) results in nitrogen losses, as ammonia (Gray *et al.*, 1971). The effects of C: N ratios of manure decomposition are discussed in section 3.8.

3.5 Microbial Transformation of Nitrogen Compounds.

Within manure and litter, nitrogen transformations are of great importance. Nitrogen transformation is normally described using the nitrogen cycle (Figure 3.7).

Figure 3.7 The Nitrogen cycle (Cornwell & Miller, 1990).



Nitrogen that is present in the manure is decomposed to ammonia (ammonification) and ammonia may also be converted to nitrate by the process of nitrification. The importance of nitrification in manure and litter is uncertain (Groot Koerkamp, 1994). This is because oxygen is mainly used by the heterotrophic microorganisms for degradation processes and is therefore barely available for the autotrophic microorganisms that are involved in the nitrification process. Neither manure nor litter

contains significant quantities of nitrate or nitrite (Groot Koerkamp, 1994). Nitrate, if present may be de-nitrified in waterlogged, anaerobic conditions (Cornwell & Miller, 1990).

The incorporation of ammonia into new N-containing organic compounds (e.g. proteins) by bacterial activity (assimilation or immobilisation) diminishes the amount of ammonia available for volatilisation (Groot Koerkamp, 1994). In addition to sufficient water, C:N ratios of at least 30 are necessary for this process (Groot Koerkamp, 1994). These ratios are not present in poultry manure and litter, so large quantities of a carbon source, such as straw, are required during the decomposition process to achieve a significant reduction in nitrogen loss (Kirchmann & Witter, 1989).

3.6 Aerobic and Anaerobic Decomposition of Manure.

Protein decomposition under anaerobic conditions indirectly leads to the release of amino nitrogen as ammonium / ammonia. Amines are initially produced and upon oxidation they are converted to ammonium / ammonia (Stanier *et al.*, 1987). Kirchmann & Witter (1989; 1992) and Kirchmann & Lundvall (1998) described an increase in ammonium ions in anaerobically decomposed poultry manure and these ions remained within the manure due to the acidic conditions present (pH 5.0-6.2). The pH of the manure has a marked effect on ammonia release as it controls the ammonium: ammonia equilibrium (Section 4.2.1). Alkaline conditions are prevalent in aerobically decomposing poultry manure as a result of microbial ammonia production (Kirchmann & Witter, 1989; 1992). This can result in nitrogen losses in the range of 44-77% of the initial nitrogen present (Kirchmann & Lundvall, 1998; Kirchmann & Witter, 1989 & 1992). Both Kirchmann & Witter (1989; 1992) and Kirchmann & Lundvall (1998) unexpectedly found that anaerobically decomposing manure lost 8% of its initial

nitrogen present over a 7 month period. This immobilisation of nitrogen in poultry manure with straw amendments was also inhibited by anaerobic conditions (Kirchmann & Witter, 1989).

Nitrogen is bound mainly in organic forms in aerobically decomposed manure, whilst in anaerobic manure it is present as ammonium ions (Kirchmann & Lundvall, 1998; Kirchmann & Witter, 1989). Therefore, the ammonium ions remain bound within the substrate and nitrogen conservation is achieved due to the acid conditions caused by the accumulation of volatile fatty acids produced within anaerobically decomposing manure (Kirchmann & Witter, 1989).

A loss of carbon (as carbon dioxide) occurs from both aerobically and anaerobically decomposing poultry manure (58% and 44%, respectively; Kirchmann & Witter, 1992). These losses occur primarily from the water-soluble portion of the total carbon content (Table 3.4). Calculation of the water-soluble C:N ratio (19.7, 6.0 & 0.3 for fresh, aerobic and anaerobic poultry manure, respectively) shows that there is a lack of water soluble carbon that would account for the high nitrogen losses in aerobic manure (Gray *et al.*, 1971) and in anaerobic manure the accumulation of ammonium ions (Kirchmann & Lundvall, 1998; Kirchmann & Witter, 1992 & 1989). The ammonium ions will be available to microorganisms that may be decomposing the organic carbon portion of the manure, whilst held within the anaerobic manure substrate.

Real world manure storage may involve both aerobic and anaerobic conditions within the manure at the same time (Kirchmann & Witter, 1989), as a result of poor structure or as a result of oxygen depletion in a specific area (Groot Koerkamp, 1994). High moisture contents in poultry manure and litter have also been cited as the main cause of anaerobic conditions in poultry manure and litter (Stentiford, 1996; Miller, 1989; Schefferle, 1965a).

Table 3.4 Characteristics of poultry manure before and after a 7 month, aerobic and anaerobic storage period (Kirchmann & Witter, 1992).

		Poultry Manure		
		Stored for 7 Months		
		Original Composition	Aerobic	Anaerobic
Ash (mg g ⁻¹ dry matter)		23.6	42.9	34.7
pH		5.9	7.9	7.8
Organic Matter (mg g ⁻¹ ash-free dry matter)	Organic Carbon	492.4	478.1	452.1
	Organic Nitrogen	61.8	40.8	25.2
	C/N _{org}	7.9	11.7	17.9
Water-soluble (mg g ⁻¹ ash-free dry matter)	Carbon	147.6	60.9	20.7
	Nitrogen	7.5	10.1	73.2
Organic Matter Losses (%)		-	57.9	40.1
Carbon Losses (%)		-	58.0	43.7
Nitrogen Losses (%)		-	76.5	7.5
Total Nitrogen (mg g ⁻¹ dry matter)		51.0	23.8	67.3
Percentage of Total – N	Ammonium – N (%)	8.2	1.5	75.6
	Nitrate – N (%)	0	0.5	0
	Urea – N (%)	1.3	0	0
	Uric Acid – N (%)	60.7	0	0
	Organic – N (%)	91.7	98.0	24.4
	Water Soluble – N (%)	70.0	24.2	71.0

3.7 Composting.

Composting is an aerobic process, which stabilises an organic substrate (Miner *et al.*, 2000; Haug, 1993). Composting is increasingly recognised as a viable treatment method for animal manure (Tiquia & Tam, 2002), as it improves its physical condition and handling characteristics. Composting begins with an initial microbially-active phase during which easily degradable substrates, such as sugars, starches and amino acids, are decomposed (Witter & Lopez-Real, 1987). This phase is characterised by increasing temperatures which occur as a result of biological heating (Haug, 1993). This phase may last from 24 hours to several weeks (Witter & Lopez-Real, 1987) and includes a period of sanitation whilst temperatures exceed 55°C (Stentiford, 1996). Maturation is the second phase and it is characterised by the formation of humic compounds (Witter & Lopez-Real, 1987). A more resistant organic fraction known as humus is produced and a degree of stabilisation occurs as the readily degradable organic compounds are oxidised by microbes and the microbes themselves die and are consumed by other microbes (Haug, 1993). Although there is not a single clear method for determining compost stability, various stability indexes and assays can be integrated to produce a reliable evaluation of compost stability (Barberis & Nappi, 1996). Monitoring temperature decline is a commonly used method to establish an end-point for composting. Once ambient temperatures are reached, the compost is considered finished (Peirce *et al.*, 1998), provided this temperature drop is not caused by microbial inactivation resulting from low moisture or low oxygen levels. This stabilisation of the end product is important as the application of un-stable immature products to soils can have a negative effect on crop development and can cause nitrogen immobilisation (high C/N ratio) or nitrogen toxicity (low C/N ratio) (Barberis & Nappi, 1996; Haug, 1993; Golueke, 1992).

Composting differs from static manure or litter storage due to the management systems (e.g. aeration and turning) involved. Manure storage without management could take many more months to reach a stable level and without turning and additional aeration may produce a non-uniform product (El-Ahraf & Willis, 1996). This is especially the case where additions are made to the composting material on a regular basis (e.g. deep-pit manure storage).

Poultry manure composting (no bulking material added) was examined by Elwell *et al.* (1998). They found the high initial moisture content of the manure could be problematic, but could be overcome by some drying on the collection belts. The composting process produced a granular material with a dry matter in excess of 800g/kg. However, when this material was re-wetted the composting process continued showing that the final product produced by their system was still un-stable. This incomplete composting may have occurred due to lack of water due to drying or lack of oxygen, which Elwell *et al.* (1998) reported to be almost depleted after only 24 hours of turning.

The composting of poultry litter is possible without amendment and can be achieved in static piles (Tiquia & Tam, 2002) or in-situ (Miller, 1999). In-situ composting can involve the reuse of litter for 5-7 consecutive flocks of broilers, with turning and levelling between flocks (Miller, 1999). This process produces a highly uniform product (properties listed in Table 3.5). The nitrogen content of the finished poultry litter compost is more stable with less than 0.5% of the nitrogen present as ammonium ions (Miller, 1999) compared to 30-50% in single-use broiler litter (Nicholson *et al.*, 1996). The nutrient contents of un-treated manure and litter and composted manure and litter are compared in Table 3.5.

Table 3.5 Properties of composts produced from poultry manure and litter compared to un-treated manure and litter.

	Composted Poultry Litter	In-Situ Poultry Litter Compost	Composted Chicken Manure ¹	Broiler Chicken Litter	Caged Laying Hen Manure (Deep-Pit)
	Dry Weight	Fresh Weight	Not Stated	Fresh Weight	Fresh Weight
pH	7.07	-	9.1	8.2	8.2
Total Carbon (g/kg)	445	-	312.2	348*	273*
Total N (g/kg)	13.65	50	47.2	33	21
Total P (g/kg)	15.38	45	-	11	7
Total K (g/kg)	17.55	40	-	16	9
Organic N (g/kg)	9.82	-	-	-	-
Ammonium N (g/kg)	3.83	-	-	6	8
C/N Ratio	32:1	-	6.67:1	6.4:1	4.7:1
Reference	Tiquia & Tam (2002)	Miller (1999)	Elwell <i>et al.</i> (1998)	Nicholson <i>et al.</i> (1996)	Nicholson <i>et al.</i> (1996)

1. No amendment added.

* Organic carbon on a dry weight basis.

In a nitrogen-rich composting substrate, such as poultry manure, there is likely to be a rapid accumulation of ammonium ions, which may result in a rise in pH (Witter, 1986). These factors combined may lead to high ammonia losses (Witter & Lopez-Real, 1987). This was the case during the composting of the un-amended poultry manure, in which Elwell *et al.* (1998) reported much greater ammonia losses than from conventional composting operations. The loss of nitrogen during the composting of a range of organic materials can result in up to 70% losses (Witter & Lopez-Real, 1987).

3.8 Carbon: Nitrogen Ratio.

The carbon: nitrogen (C:N) ratio is critical to the decomposition characteristics of organic materials. Carbon is the major energy source for microorganisms and nitrogen is essential for growth (Haug, 1993; Golueke, 1992) (Section 3.4.6). However, carbon and nitrogen which are present within the organic wastes in excess to microbial requirements, are released as carbon dioxide and ammonium / ammonia, respectively (Higgins & Burns, 1975).

A C: N ratio of 20-30:1 is optimal for most composting or decomposition processes (Haug, 1993; Golueke, 1992). At levels below 15:1, mineralization of nitrogen exceeds microbial immobilisation of nitrogen (Haque & Vandepopuliere, 1994) and nitrogen is largely lost as ammonia (Haug, 1993; Gray *et al.*, 1971). However, almost all of the nitrogen can be retained within high C:N ratio materials. In these situations, microbial reproduction and growth would decline in proportion to the loss of nitrogen in the substrate (Golueke, 1992). C: N ratios that exceed 30:1 have a carbon surplus and so emit carbon dioxide (Gray *et al.*, 1971) and only small nitrogen losses would occur in these substrates (Miner *et al.*, 2000).

Eventually composts with a high C:N ratio reach a lower ratio as a result of microbial respiration and growth. Two thirds of the carbon consumed by microorganisms is evolved as carbon dioxide and one third is combined with nitrogen in the living cell (Golueke, 1992). Upon the death of successive microbial communities, fixed nitrogen and carbon become available, and two thirds more of the available carbon is again evolved as carbon dioxide. Thus, the amount of carbon is reduced and nitrogen is recycled through subsequent generations (Atkinson *et al.*, 1996; Golueke, 1992). These effects are demonstrated by Flynn & Wood (1996) and Kirchmann & Witter (1989) in

poultry litter composting and poultry manure storage, respectively. There was a greater loss of carbon than nitrogen from the decomposing litter and manure in both studies. The low pH of the poultry litter composts (5.5-7) also indicated that carbon dioxide production exceeded ammonia production (Haug, 1993; Witter & Kirchmann, 1989b).

The low C: N ratios found in poultry manure (<10-15:1) provide insufficient carbon for adequate microbial metabolism and so the microbial populations utilise nitrogen-containing compounds for their carbon requirements and release un-used nitrogen as ammonia (Golueke, 1992). Nitrogen losses of 40% may occur with C: N ratios of 20:1 (Miner *et al.*, 2000). C:N ratios should be expressed using available carbon and nitrogen data (Haug, 1993), as carbon bound in resistant forms (e.g. cellulose) that are not readily degradable by microbial attack, are not able to be utilised along with available nitrogen sources (Golueke, 1992).

4. Emission of Ammonia from Poultry Manure and Litter.

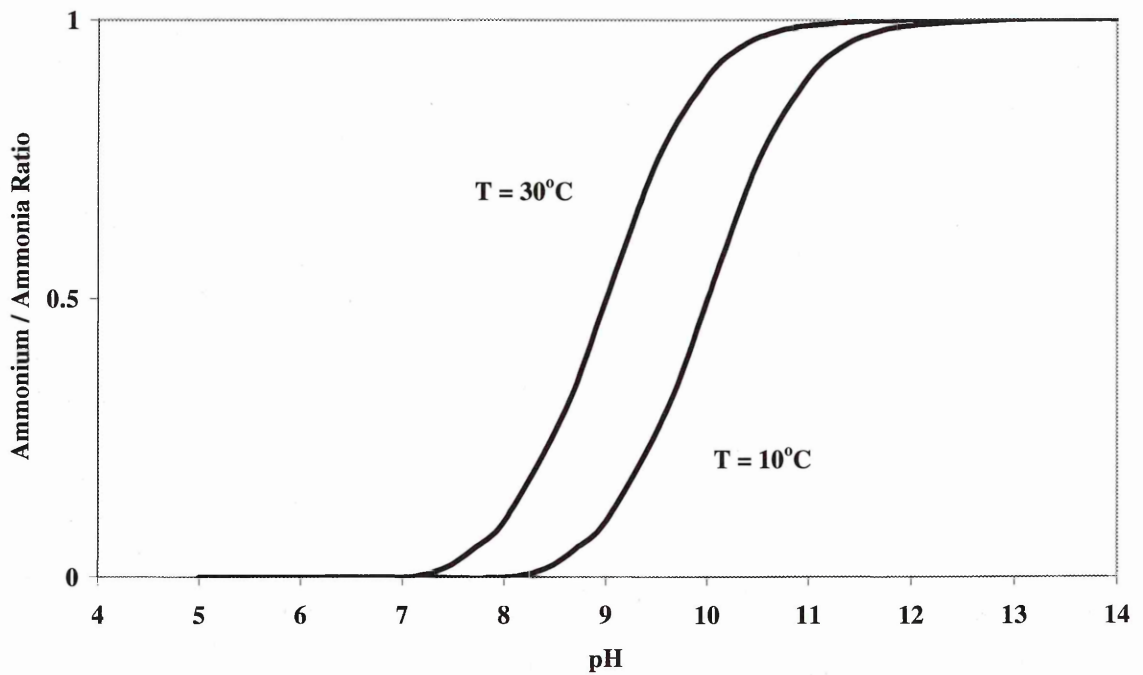
Ammonia that is released from poultry manure or litter substrates is present as ammonium compounds, aqueous ammonia and the positively charged ammonium ion (Elliott & Collins, 1982). These species together can be described as the total ammoniacal nitrogen pool (TAN pool). The TAN pool is susceptible to volatilisation losses, unless it is immobilised by its assimilation into the microbial biomass, its formation into chemical compounds or being attached to humic materials (Witter, 1986). Since the aqueous form of ammonia is a volatile base, an equilibrium will be established between soluble ammonia in the litter or manure and the gaseous ammonia in the litter or manure air space (Elliott & Collins, 1982). Thus, the TAN pool is subject to a series of equilibria, involving ammonia in these liquid, gaseous and ionised forms, before it is released into the atmosphere.

These equilibria are greatly affected by pH and temperature (Sections 4.2 & 4.3 respectively). Figure 4.1 shows the relationship between pH and the proportion of ammonia and ammonium ions at two aqueous solution temperatures (Groot Koerkamp, 1994).

The volatilisation of ammonia occurs as a result of its evaporation from the manure solution according to Henry's Law for dilute systems (Equation 4.1). Where P_{NH_3} is the partial pressure of ammonia in the manure, NH_3 (aq) is the aqueous ammonia in the manure and K_{H} is the Henry's Law constant (Fowler *et al.*, 1996; Elliott & Collins, 1982), where the concentration of a gas in solution is proportionally dependent on its partial pressure (Kruse, 1986).

$$4.1 \quad P_{\text{NH}_3} = \text{NH}_3 \text{ (aq)} / K_{\text{H}}$$

Figure 4.1 The dependence of ammonium / ammonia ratio on pH and temperature (T) (Groot Koerkamp, 1994)



The volatilisation of ammonia from the manure to the air is defined as the mass flux (Equation 4.2) (Groot Koerkamp, 1994). The rate of this transfer (NH_3A – aerial mass flux of ammonia released from the manure surface) depends upon the mass transfer coefficient (K_G) and the difference between the manure and atmospheric partial pressures ($P_M - P_A$) (Elliott & Collins, 1982). The greater the difference between P_M and P_A the greater the value of the mass flux of ammonia from the surface of the manure.

$$4.2 \quad \text{NH}_3\text{A} = K_G (P_M - P_A)$$

The partial pressure of ammonia in the manure (P_M) is large (1-20 ppmv – Fowler *et al.*, 1996) compared to the partial pressure of ammonia in the house or local atmosphere (P_A) (0.01-10 ppb – Fowler *et al.*, 1996). Therefore, ammonia will be transferred from

the manure to the atmosphere (Kruse, 1986; Elliott & Collins, 1982). Kruse (1986) concluded that the partial pressure of ammonia in solution was determined by the hydrogen ion concentration (e.g. pH), the ammonium ion concentration and temperature. The partial pressure of ammonia within the manure is also increased as more ammonia / ammonium is produced by microbial activity within the manure.

4.1.1 Volatilisation of water.

The removal of water from poultry manure and litter is desirable to reduce the volume of manure or litter to be handled. The evaporation rate of water can be increased by increasing temperature and air flow, and by decreasing the water vapour pressure. This directly conflicts with the factors that aim to prevent ammonia release from manure (Groot Koerkamp *et al.*, 1998). The removal of water from the manure or litter substrate also has an effect on ammonia volatilisation. If the same amount of ammonia is dissolved in less water, the partial pressure of ammonia in the manure increases and volatilisation is increased (Carr *et al.*, 1990). However, as ammonia is very soluble in water, volatilised ammonia may dissolve in the water vapour (Valentine, 1964) and be removed via the ventilation system or may be condensed on a cold surface and remain within the housing system. In either case, the partial pressure of ammonia in the air is reduced and ammonia volatilisation from the manure or litter continues. Therefore, the removal of water from the manure / litter can promote ammonia volatilisation. However, this will not continue indefinitely as the removal of water from the manure will reach critical levels for microbial activity and hence decomposition will slow or even stop. This effect of water loss is discussed in section 3.4.3.

The volatilisation of water from manure or litter can reduce ammonia volatilisation once critical water levels are achieved. This is achieved by rapid drying in order to reduce

ammonia production and volatilisation during the process, though in practice drying systems are not common in the U.K poultry industry. Drying systems are discussed in section 5.1. The removal of both water and ammonia in contaminated air by ventilation systems affects the partial pressure of the ammonia and water allowing the further release of these gases from the manure or litter substrate.

4.1.2 Ventilation Rate.

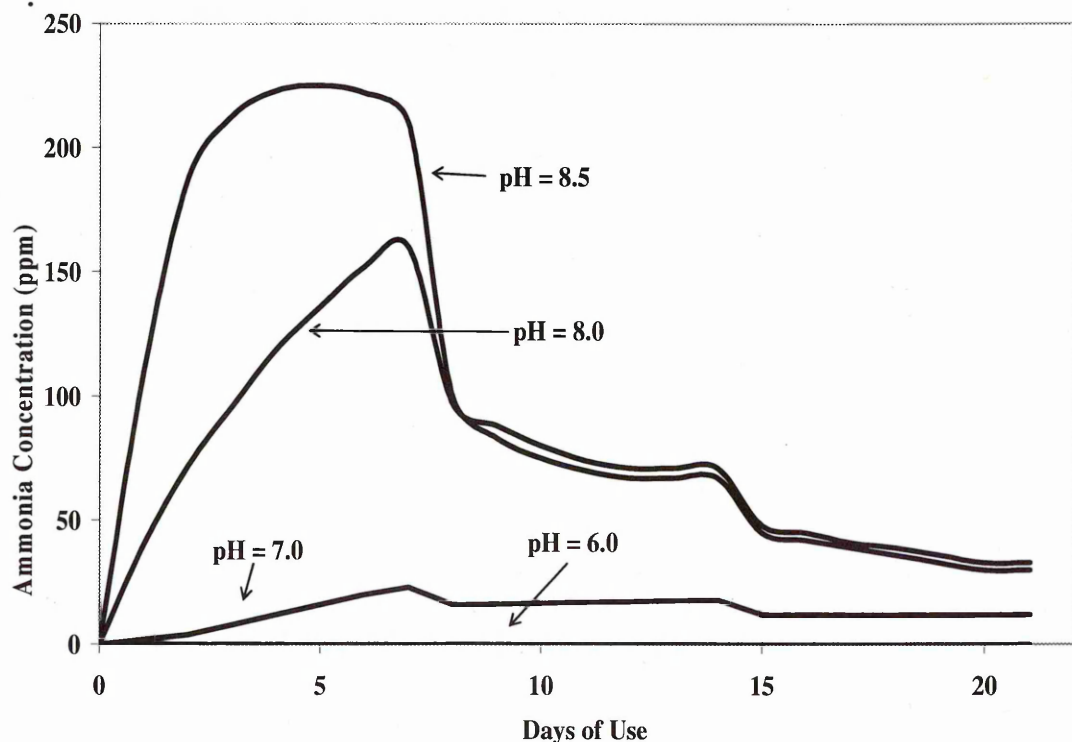
The ambient gaseous ammonia level in a housing system is equal to the rate of volatilisation from the manure or litter surface minus the rate of ammonia removal in the exhaust air (Elliott & Collins, 1982). Thus, ventilation rates have a profound effect on the rate of ammonia volatilisation. Ammonia concentrations within a housing system are decreased as ventilation rates increase (Carr *et al.*, 1990; Van Wicklen & Allison, 1989). The ammonia mass transfer coefficient is increased with increasing air velocity over the surface of the manure (English *et al.*, 1980).

Ventilation rates are usually designed with regard to temperature control, for relative humidity control (Esmay, 1978; Maghirang *et al.*, 1991) and possibly ammonia levels. Ventilation rates that are designed to minimise ammonia levels are likely to be higher than those required for temperature or relative humidity control. In order to reduce ammonia levels, Elliott & Collins (1982) increased ventilation rates by ten times the normal rate, which gave a ten fold decrease in ammonia levels but significantly increased heating costs. The increased ventilation rates not only increased fuel usage and bird feed intakes (Carr & Nicholson, 1980), but also increased aerial dust and particulates as a result of drier litter / manure (Maghirang *et al.*, 1991). Alternative methods of controlling ammonia levels in poultry houses need to be investigated (Maghirang *et al.*, 1991; O'Connor *et al.*, 1988).

4.2 Effect of pH.

The pH is important in the control of atmospheric ammonia release from poultry litter and manure (Reece *et al.*, 1979; Elliott & Collins, 1982; Carr & Nicholson, 1980). In poultry litter, the bacterial decomposition of uric acid results in a rise in pH, as a result of ammonia production (Elliott & Collins, 1982; Schefferle, 1965a, 1965b; Turnbull & Snoeyenbos, 1973).. There is a positive relationship between the ammonium nitrogen concentration of poultry manure and the pH value of the manure (Nicholson *et al.*, 1996). Thus, it is an important factor influencing ammonia volatilisation (Elliott & Collins, 1982). The effect of reducing pH in litter from a normal 8.5 to a lower pH is illustrated in Figure 4.2. This effect displays the benefits of preventing the pH from rising to alkaline values (Elliott & Collins, 1982). These effects have also been observed by Reece *et al.* (1979).

Figure 4.2 The effect of litter pH on ammonia levels (Elliott & Collins, 1982).



The strong influence of pH is a reflection of its effect on two steps of the volatilisation process, first bacterial ammonification and second gaseous ammonia production as pH alters the ammonia holding capacity of the litter (Elliott & Collins, 1982).

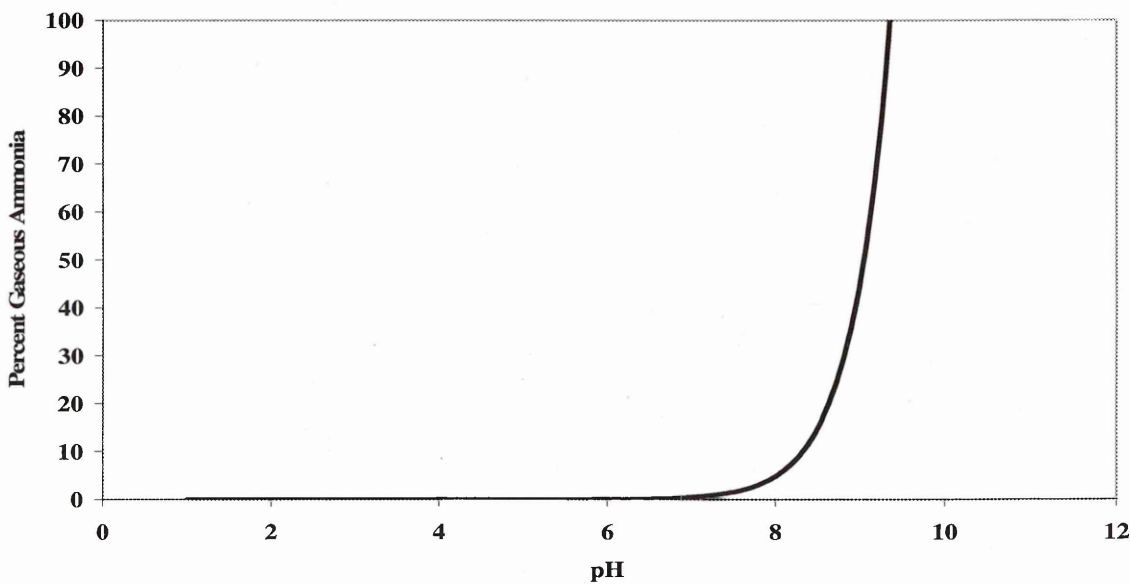
Attempts to experimentally control pH chemically in order to reduce ammonia volatilisation from broiler litter have been unsuccessful (Carr *et al.*, 1990). A non-linear model was developed to predict pH as a function of house temperature, relative humidity and litter moisture content (Carr *et al.*, 1990). Using this model, Carr *et al.* (1990) examined ammonia concentrations with respect to a number of independent variables. They showed that atmospheric ammonia concentrations increased when pH increased, with relatively little ammonia production at pH values below 7.5 (Carr *et al.*, 1990). Above this value emission rates of ammonia increased exponentially and a combined increase of litter pH and house temperature produced a far greater increase than from a single variable (Section 4.5).

Since ammonia is alkaline, its production causes a localised increase in pH, causing the majority of the ammonical nitrogen to be in the form of ammonia rather than ammonium ions (Fowler *et al.*, 1996). The percentage of ammonia in the total ammonical nitrogen pool at (25°C) as a function of pH is shown in Figure 4.3.

The effect of pH on ammonia volatilisation are also relevant to work on fertiliser applications to acidic and alkaline soils (Witter, 1986). For example, 87% of fertiliser nitrogen was volatilised at pH 10.5 compared to 13% at pH 8.6, whilst no volatilisation was detected at 7.0 (Jewitt, 1942). The manure pH is greatly influenced by the ratio of microbial carbon dioxide and ammonia production (Witter & Kirchmann, 1989b). Carbon dioxide production will lead to net acidification (Witter & Kirchmann, 1989b), while the rapid microbial transformation of urea to ammonium carbonate, will cause a rise in the ambient pH (Kruse, 1986; Vlek & Stumpe, 1978), resulting in ammonia

volatilisation. In the absense of other sources of alkalinity, significant ammonia losses can therefore only be expected when ammonia production significantly exceeds carbon dioxide production (Witter & Kirchmann, 1989b). As the loss of ammonia is also associated with an equivalent loss of alkalinity (Vlek & Stumpe, 1978) an equilibrium is always reached between ammonia and the ammonium ion with respect to pH.

Figure 4.3 The percentage of gaseous ammonia in the total ammoniacal nitrogen pool at (25°C) as a function of pH, calculated from data presented in Kirchmann & Witter (1989).



4.2.1 Ammonium : Ammonia Equilibrium.

The TAN pool can be partitioned into ammonium ions and ammonia at a constant partial pressure. As pH is a logarithmic function, lowering the pH by one unit increases the ammonia holding capacity of the litter tenfold (Elliott & Collins, 1982). Therefore, the ammonia concentration within a building will be reduced markedly if the pH is reduced from 8.5 to 6.0 as the litter is capable of holding over 300 times the TAN (as

ammonium ions) for the same ambient ammonia partial pressure in the house atmosphere (Elliott & Collins, 1982).

The ammonium / ammonia equilibrium is a complex system that is affected by a number of factors that include temperature and partial pressure, but most importantly pH. As pH may be continually changing as a result of ammonia loss (Vlek & Stumpe, 1978) or production (Fowler *et al.*, 1996), the equilibrium is in a state of continual flux.

4.3 Effects of Temperature.

Models created by Carr *et al.* (1990) and Elliott & Collins (1982) show an increase in ammonia volatilisation with elevated temperatures. This occurs for a number of reasons. First, temperature has a positive influence on the dissociation constant (K_a) (Equation 4.3) which shifts the ammonia / ammonium equilibrium in favour of ammonia.

$$4.3 \quad K_a = [\text{NH}_3] [\text{H}_3\text{O}^+] / [\text{NH}_4^+]$$

Secondly, ammonia volatilisation is increased due to the effect of temperature on the partial pressure of ammonia (Kruse, 1986). Higher temperatures raise the partial pressure of ammonia, a temperature rise of 20-30°C decreases the Henry constant (Equation 4.1) by about half (Groot Koerkamp, 1994). For any given concentration of ammonia within the manure solution, the volatilisation of ammonia would be doubled due to a similar increase in the partial pressure of ammonia.

Also, increasing the temperature will increase the rate of microbial proliferation and hence the degradation of the manure or litter so this increases the size of the TAN pool from which the ammonia is volatilised (Elliott & Collins, 1982). Finally, increasing temperature increases the mass transfer of ammonia in the liquid phase to the gas phase.

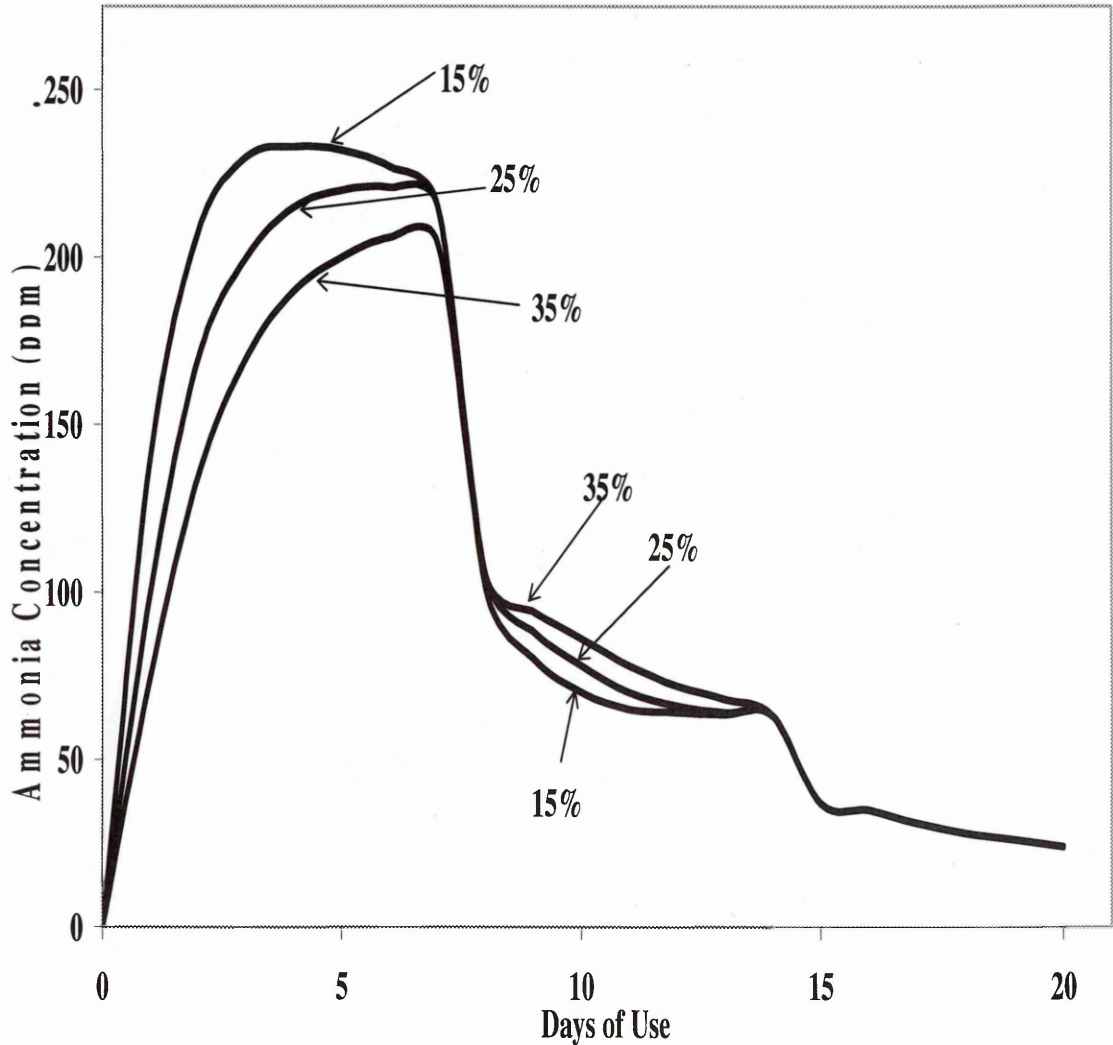
Thus, because temperature affects several steps in the volatilisation process, relatively small increases in temperature can yield substantial changes in ambient ammonia levels (Elliott & Collins, 1982). Carr *et al.* (1990) showed that there was an exponential increase in ammonia concentrations when temperatures exceeded 25°C.

4.4 The Effects of Water Content.

The moisture content of manure has less influence on ammonia volatilisation than pH and temperature (Elliott & Collins, 1982). Both very high and very low moisture contents reduce ammonia volatilisation, but differences in moisture content give both increases and decreases in ammonia volatilisation. Elliott & Collins (1982) showed a reduced level of ammonia volatilisation at higher moisture contents in the first week of manure storage, but in subsequent weeks (Figure 4.4) the higher moisture contents give a higher ammonia volatilisation.

In principle, the same amount of ammonia dissolved in increasing volumes of water results in lower concentrations of ammonia, and hence gives reduced volatilisation rates (Elliott & Collins, 1982). These differing volatilisation rates affect the TAN pool and the lower moisture contents become a constraining decomposition factor during the following weeks, hence the amount of ammonia available for volatilisation in subsequent weeks is reduced. Increases in moisture content generally produce a large increase in ammonia concentration (Figure 4.5 – Carr *et al.*, 1990), which have been attributed to greater capillary action in the manure, which increased the rate of diffusion into the atmosphere.

Figure 4.4 The influence of moisture content on ammonia levels (Elliott & Collins, 1982).



Decreases in ammonia concentrations at high moisture levels have been reported by Valentine (1964) and Schefferle (1965b). This suppression of ammonia release is thought not only to relate to the dilution effect highlighted by the model of Elliott & Collins (1982), but also to be due to the anaerobic conditions created by high moisture contents vastly reducing the activity of the predominantly aerobic populations that are considered to be responsible for ammonia production (Carr *et al.*, 1990; Schefferle, 1965b; Valentine, 1964). The evaporation of water is increased by both increases in

temperature and aeration. As the evaporation of water from the manure increases, the loss of ammonia (that is dissolved in the water) is increased linearly (Maeda & Matsuda, 1997). Hence, ammonia is not only lost in gaseous form but also lost whilst dissolved in the evaporating water.

4.4.1 Relative Humidity.

High levels of relative humidity indicate high levels of moisture present in the air, relative to temperature and pressure. Gaseous ammonia is highly soluble and so dissolves in the moisture (forming ammonium ions) and this reduces the gaseous ammonia concentration in the air. Thus, high ammonia concentrations are more likely at low relative humidity (O'Connor *et al.*, 1988). However, Valentine (1964) concluded high relative humidity resulted in high ammonia concentrations in commercial poultry units, as no distinction was made between gaseous ammonia, aqueous ammonia and ammonium carbonate in the moist air. This confirms the theory that gaseous ammonia dissolves or reacts with other components of the air, as Valentine (1964) found the relative humidity was closely related to ammonia concentration measurements.

It should be noted that the direct measurement of gaseous ammonia will not account for all ammonia losses, as it is a reactive gas that is quickly removed from the atmosphere by chemical reactions (Section 2). Methods that involve dissolving air samples into solutions prior to analysis are likely to give a more accurate detection of ammonia and related compounds in the air sample.

4.5 Combination of Factors.

The combination of effects such as temperature, litter moisture and ventilation rates on ammonia concentrations (independent of pH) are shown in Figure 4.5. An interaction

between temperature and relative humidity should be expected, as these factors do not vary independently. An interaction between temperature and litter moisture is also expected, because evaporation is dependent on the water holding capacity of the air, which is a function of temperature and humidity (Carr *et al.*, 1990). Also, the higher the temperature the more energy is available for the evaporation of water. However, at high litter moisture contents and high temperatures there was a decrease in ammonia concentrations. This was reported to be due to a negative interactive term between temperature and litter moisture (Carr *et al.*, 1990). As previously discussed (Section 4.4), high moisture levels have been reported to suppress ammonia concentrations and these data would indicate that this effect is increased at high temperatures.

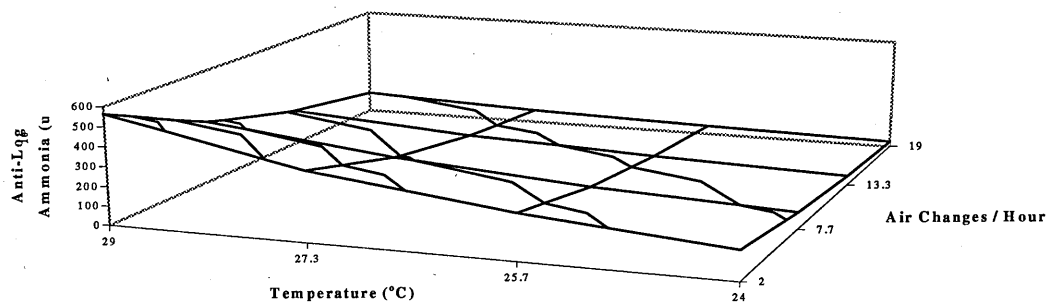
The pH level of the manure is also an important consideration in the release of ammonia. The interaction of pH with the environmental variables, temperature and ventilation rate is shown in Figure 4.6. The exponential effect of pH on ammonia volatilisation is increased when temperature increases.

4.6 Other factors that affect ammonia volatilisation.

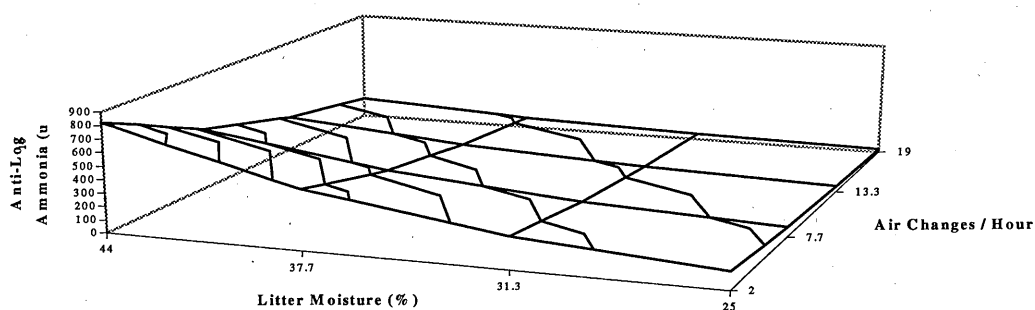
The volatilisation of ammonia from manure or litter is also affected by factors such as the surface area of the manure heap in contact with the air, and the length of time the manure is in contact with the air (Groot Koerkamp, 1994).

Figure 4.5 The interaction between temperature (T), litter moisture content (LM) and ventilation rate (AC) on the volatilisation of ammonia (NH₃). Graphs drawn using the model proposed by Carr *et al.* (1990).

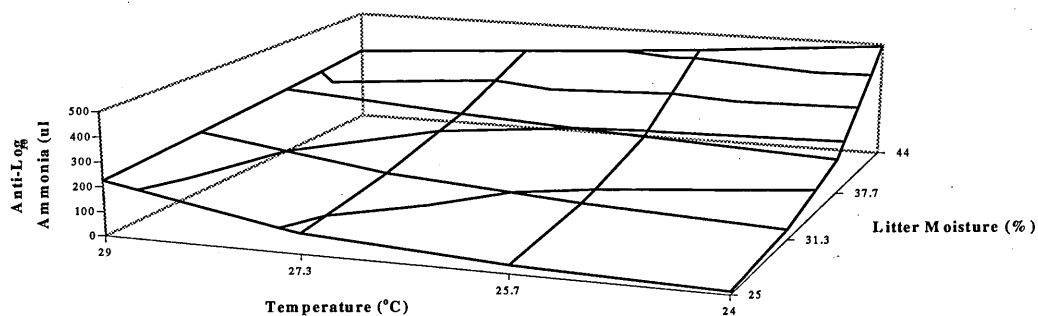
$$\text{Log}_{10} \text{NH}_3 = (0.623 \times T) - (0.042 \times \text{AC}) + (0.443 \times \text{LM}) - (0.015 \times T \times \text{LM}) - 15.503$$



A Response surface plot of ammonia concentration, with litter moisture held constant at 33.87%



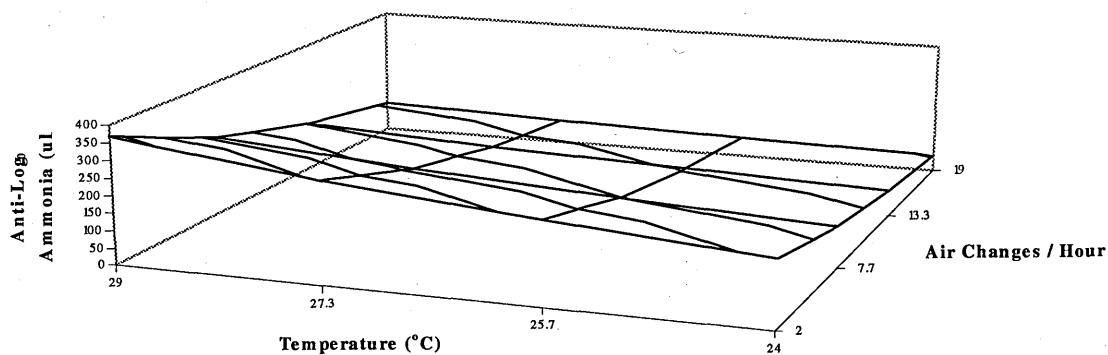
B Response surface plot of ammonia concentration, with temperature held constant at 26.75°C.



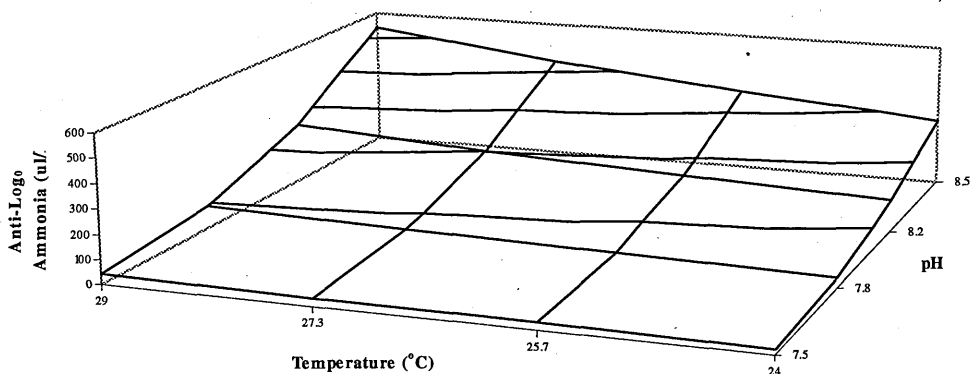
C Response surface plot of ammonia concentration, with ventilation rate held constant at 9.8 air changes per hour.

Figure 4.6 The interaction between temperature (T), pH (pH) and ventilation rate (AC) on the volatilisation of ammonia (NH₃). Graphs drawn using the model proposed by Carr *et al.* (1990).

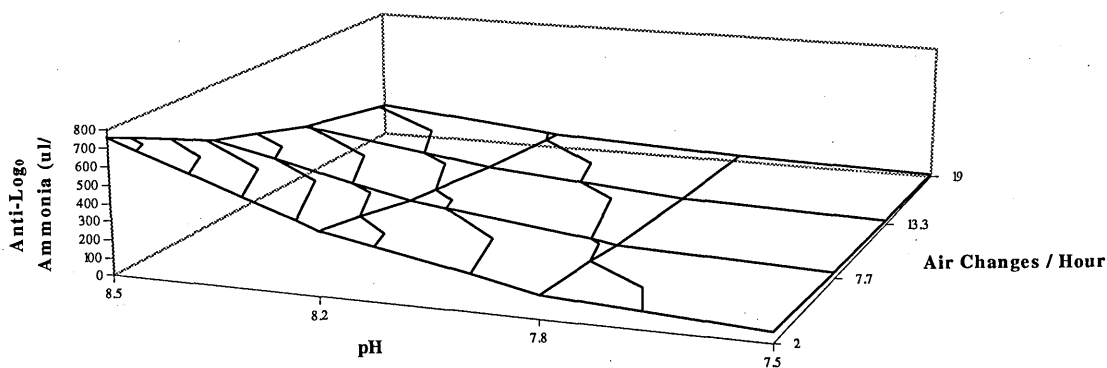
$$\text{Log}_{10} \text{NH}_3 = (1.089 \times \text{pH}) + (0.056 \times T) - (0.035 \times \text{AC}) - 7.811$$



A Response surface plot of ammonia concentration, with pH held constant at 8.1.



B Response surface plot of ammonia concentration, with ventilation rate held at 9.8 air changes per hour.



C Response surface plot of ammonia concentration, with temperature held constant at 26.87°C.

5. Manipulation of Housing Design and Management to Control Ammonia Emissions.

The control of ammonia emissions from poultry production systems can be achieved by careful consideration of production methods. Efficient diet formulation can reduce ammonia emissions by reducing the crude protein content, normally accompanied by amino acid supplementation (Hobbs *et al.*, 1997) and minimising inclusion of excess minerals (e.g. sodium, potassium, calcium and phosphorus), that will reduce the moisture content of the excreta (Smith *et al.*, 2000a & b). However, as diets are formulated to maximise production, changes in the diet are likely to increase costs and may reduce production targets (Groot Koerkamp, 1994).

Housing design and management can also have a significant effect on ammonia emissions. There are two major types of poultry housing; floor based systems and cage systems (Mercia, 2001). Floor systems may consist of litter, slats or wire mesh. Approximately 66% of the edible eggs produced in the U.K. are from laying hens housed in cage systems. The remainder are from hens housed in free-range systems (27%), barn or other systems (7%) (DEFRA, 2005). Litter based systems produce much higher ammonia emissions than cage systems (Appleby & Hughes, 1991). Attempts to reduce ammonia emissions from litter systems can include floor insulation (40% reduction, Ouwerkerk & Voermans, 1986), floor heating (up to 40% reduction, Dobrzański & Białas, 1993) and litter ventilation (50-90% reduction, Macke & Van den Weghe, 1997; Middelkoop *et al.*, 1994). All of these were successful by improving litter quality through an increase in dry matter. Good management of water systems also helps reduce ammonia volatilisation (Phillips & Chambers, 2002).

Some cage systems have sufficient storage space for the manure to remain within the house for the whole production cycle, whilst some systems have belts underneath the cages which collect manure and can be run off to remove the manure at intervals. Slatted floor systems can also store manure within the housing structure for limited periods, whereas barn systems tend to retain manure until the end of the production cycle. Additional litter may be used to adsorb moisture and thus retain litter quality.

Irrespective of the type of housing system, there are four main methods of manure removal and storage, though most systems are likely to use more than one system of manure removal.

1. Manure falls immediately into storage area.
2. Manure is temporarily stored on belts or trays (with or without manure drying) and removed to a storage area within the house.
3. Manure is temporarily stored on belts (with or without manure drying) and removed to a storage area outside the house.
4. Manure is incorporated into litter-based bedding.

Aspects of managing these systems can have a profound effect on ammonia emissions and are discussed in the following sections.

5.1 Using Manure Handling to Control Ammonia Emissions.

5.1.1 Belt Removal.

Manure collection on belts beneath cages has both advantages and disadvantages. Immediately following excretion, the nitrogen contained in the egesta and excreta is liable to volatilisation as ammonia (Chang & Flint, 1976). Storage of the manure

directly below the hens can cause high ammonia concentrations within the stocked area. However, it may be beneficial to hold manure on the belt at this point as the higher temperatures and airflow rates will facilitate drying of the manure (Kroodsma 1985 & 1986), so reducing its volume and making it easier to handle. But even when forced air drying of the manure on the belt occurs (Section 5.1.5), a significant quantity of ammonia is lost during this period (Van Horne, 1997).

The period of storage of manure on belts underneath cages is restricted to the storage capacity of the belts, but is directly related to the level of ammonia in the house (Kroodsma *et al.*, 1988).

5.1.2 Frequency of Manure Removal.

The frequency of manure removal from the stocked area varies according to the type of housing system. When there are automated manure removal belts, frequent removal of manure is possible. The manure may be moved out of the housing area into an external storage area or into a storage area within the housing system (e.g. pit or tank below the cages). Ammonia emissions from the building can be reduced by approximately 50% (ADAS, 2002) if the frequency of manure removal from the building is increased from once to twice a week, with daily manure removal producing no additional benefits. However, removal of manure from the housing system requires an additional storage area. If this is open to the environment, significant nitrogen losses may occur through both ammonia volatilisation and nitrogen leaching. Covered storage areas or sheeted manure piles are recommended for systems that do not store manure within the housing system (Phillips & Chambers, 2002).

5.1.3 Storage of Manure within the Housing System.

Manure storage within the housing system may occur when manure falls directly into the pit from the stocked area or when manure collection belts are scraped off into the pit. This method of storage has disadvantages. Commonly, in order to prevent gaseous pollutants returning to the stocked area, the ventilation system exhausts air from the house through the pit area. This causes warm air to be drawn across the surface of the manure. Though this will cause some reduction in dry matter, it is likely that the composting process will proceed within the stored manure, which also facilitates ammonia volatilisation (Section 3.7). Any increase in dry matter that occurs as a result of the pit environment is likely to be insufficient to prevent the detrimental effect of composting in terms of nitrogen loss. In order to combat this problem, the “stilt house” was developed. This is a high rise cage system, with open sided manure storage. Ammonia emissions from this type of system may be 50% of that emitted from deep-pit houses, but this estimate does not account for losses due to wind movement across the exposed surface of the stored manure (ADAS, 2002).

In order to reduce ammonia loss from stored manure within a housing system, temperature must be reduced significantly. Temperature reduction would affect the productive performance of the stock, so other measures of control must be considered such as manure amendments or air filters.

5.1.4 Storage of Manure Outside the Housing System.

Many authors cite manure removal as a way of reducing ammonia emissions from the housing system (Aono *et al.*, 1997; Rotz, 2004; Van Horne *et al.*, 1998). Though this is indeed true, the stored manure will still release ammonia. To minimise emissions manure should be stored in ‘A’ shaped heaps to keep the manure dry (Phillips *et al.*,

1999) and where possible manure should be stored in a covered yard to protect the manure from rainfall and subsequent washout of nutrients (Phillips & Chambers, 2002). The covering of manure heaps with a range of materials such as plastic, straw, woodshavings, zeolite, clay soil and compost (Burton, 1997; Witter & Lopez-Real, 1988; Filson *et al.*, 1996) has been shown to be effective in reducing ammonia volatilisation, as it restricts air movement over the surface of the manure.

The storage of manure outside the housing system requires a storage area of sufficient capacity for the whole year in case land application is restricted due to unforeseen circumstances such as adverse weather conditions etc. Ammonia control measures should be considered as the stored manure would still be biologically active.

5.1.5 Manure Drying Systems.

Manure drying has become an increasingly popular method of handling manure, because it makes it easier and cheaper to store and apply to land (Van Horne, 1997). The drying of manure whilst on belts, directly underneath cages produces dry matters in excess of 450g/kg within 4-5 days (Kroodsma, 1986). This can reduce ammonia emissions from housing systems with weekly removed belt manure to the same level as systems with twice weekly removal (Kroodsma *et al.*, 1988). However, the drying of manure to around 450-500g/kg does not halt microbial activity. Elliott & Collins (1983) state that 400-600g/kg dry matter is the optimum range for microbial growth (Figure 3.5). Hence, the subsequent storage of this dried manure is likely to produce significant quantities of ammonia (Kroodsma *et al.*, 1996). However, slight differences in the dry matter content of the manure in this range can produce substantial reductions in ammonia emissions (Kroodsma *et al.*, 1996).

Though manure drying can produce large reductions in ammonia volatilisation, the actual drying process itself not only stimulates the evaporation of water, but also the volatilisation of ammonia (Groot Koerkamp *et al.*, 1998).

5.1.6 Composting.

Elwell *et al.* (1998) reported very high ammonia emissions from composting un-amended poultry manure. Even when pre-dried poultry manure (approximately 500g/kg dry matter) was stockpiled with a passive air drying system, significant quantities of ammonia were released (52g NH₃-N per hen per year) (Kroodsmas *et al.*, 1996). Both these studies report an end product with a dry matter in excess of 800g/kg, but when the manure was re-wetted, Elwell *et al.* (1998) found the end product to be unstable and that it continued to decompose, and produce ammonia. The composting of poultry manure with carbonaceous amendments is discussed in section 6.2.4. Even with amendments, the composting of a nitrogen-rich substrate such as poultry manure and litter is likely to result in considerable nitrogenous losses (Raviv *et al.*, 1999; Elwell *et al.*, 1998; Maeda & Matsuda, 1997; Mondini *et al.*, 1996). Strict management of manure composting is required in order to minimise nitrogen loss and to ensure a well finished end product is produced.

5.2 Using Environmental Control of Housing Systems to Control Ammonia Emission.

Environmental conditions within a poultry house are designed to maintain a temperature conducive to bird production and to provide sufficient fresh air, removing moisture and pollutants for the healthy development of the stock (Rose, 1997). However, these factors conflict as the ventilation rates required to remove moisture and gaseous

pollutants often exceed those required for temperature control, especially during cold weather (Elliott & Collins, 1982). However, restriction of ventilation rates can be beneficial in reducing ammonia emissions. The ammonia concentration in the house air will reach an equilibrium with the ammonia in the stored manure and volatilisation ceases. Ammonia concentrations within the manure may then become toxic to microorganisms and ammonia production will cease (Miner *et al.*, 2000; Groot Koerkamp, 1994).

Mechanically ventilated houses can be fitted with filters to remove ammonia from the spent air. Such filters or scrubbers may remove ammonia by chemical or physical means and these methods are discussed in section 6.3.

6. Additional Methods of Controlling Ammonia Emission.

6.1 Dietary Additions.

Dietary amendments must be carefully considered as they must not interfere with the efficiency of the production process.

6.1.1 Ion Exchange.

Zeolites are crystalline, hydrated aluminosilicates of alkaline earth cations (Carlile, 1984). Their constituent cations are easily exchangeable and the zeolite, clinoptilolite, has as affinity for ammonium ions (Koelliker *et al.*, 1978). Additions of zeolites to feed to improve growth rates and to reduce ammonia and other odours have given variable results. Barrington & El Mouedebb (1995) reported reductions in ammonia emission from manure produced from feeding zeolites, but the majority of published reports found no significant effects on ammonia emissions from manure by feeding zeolite (Nakaue & Koelliker, 1981; Koelliker *et al.*, 1978; Airoidi *et al.*, 1993). Amon *et al.* (1995) reported ammonia concentrations to be 46% higher from manure produced by broiler chickens fed clinoptilolite. But increases in egg production (Olver, 1989), feed efficiency (Airoidi *et al.*, 1993) and decreases in faecal moisture (Olver, 1989; Nakaue & Koelliker, 1981) and mortality (Olver, 1989) provide some benefits from the feeding of clinoptilolite. Nakaue & Koelliker (1981) and Koelliker *et al.* (1978) reported a “fishy” odour from manures produced by hens fed clinoptilolite.

6.1.2 Enzyme Inhibition.

The addition of the saponins extracted from the *Yucca schidigera* to feeds as a growth promoter has produced some additional benefits in reducing ammonia losses. *Yucca*

saponins have an inhibiting action on urease, as well as containing two glycoproteins that bind ammonia (Section 6.2.3) (Whyte, 1993). The supplementation of *Yucca* extracts increased poultry weight gains (Davidson, 1991; Johnston *et al.*, 1981), but the effects on ammonia emission were variable. Johnston *et al.* (1981) and Amon *et al.* (1995) found no difference in ammonia levels as a result of feeding *yucca* extracts and a proprietary product (De-odorase) to broilers, respectively. Whereas, Headon & Walsh (1993) who fed pigs De-odorase showed a significant decrease in aerial ammonia levels. De-odorase is a commercially available *yucca* preparation (Alltech Ireland Ltd, Dunboyne, Co. Meath, Ireland) which has been formulated to be suitable for application via the feed or directly to manure or litter. It contains enzymes and bacterial products that promote fermentation and increase degradation of the manure, as well as the purified *yucca* sap extract (Davidson, 1991).

The urease inhibiting properties of *Yucca* extracts were utilised by Balog *et al.* (1994) to reduce broiler gut ammonia levels. This was successful, but resulted in dose response increases in the aerial ammonia concentration following excretion. Other urease inhibitors, phenyl phosphorodiamidate and n-(n-butyl) thiophosphoric triamide reduced the rate of urea hydrolysis, with 100% and 74% of urea still remaining after one day, whereas in un-treated samples no urea was left after one day (Varel *et al.*, 1997). These effects were not long lasting and regular applications were required to prevent urea hydrolysis and hence ammonia production. Another feed additive, Micro-aid (Distributors Processing Inc., Porterville, California.), which also contains a urease inhibitor, reduced the ammonia emissions from a laying hen house by 25% (Goodall *et al.*, 1988).

The addition of ammonia controlling or reducing amendments to feed, requires that the amendment does not adversely affect production targets and is sufficiently protected from the digestive process, if it is to have an effect on ammonia control in the manure.

6.2 Manure Amendments.

In caged laying hen systems and some alternative systems, there is an opportunity to add amendments to the manure to control ammonia losses. Such amendments may be broadly categorised by their mode of action and are discussed in the following sections.

6.2.1 Microbial Inhibition.

Suppression of microbial activity prevents the breakdown of uric acid and urea to ammonium / ammonia. The activity of microbes can be reduced or they can be destroyed by extremes of temperature, moisture or pH and reduced oxygenation. Inhibition of microbial activity can be achieved chemically or by manipulating the physical environment.

Temperature and moisture effects can be utilised through forced air drying of the manure (Section 5.1.5) which can reduce or prevent microbial activity. This practice can manipulate moisture conditions, but extremes of temperature are more difficult to apply in practical situations. However, it is known that heating a substrate to 55°C for four hours is sufficient for sludge pasteurisation (DoE, 1989). The addition of various chemical compounds to the stored manure or litter to reduce or prevent microbial ammonia production is discussed below.

Paraformaldehyde (a disinfectant) not only combines with ammonia (Elliott & Collins, 1983), but also has an anti-microbial effect. When it was applied to broiler litter at 3%

(w/w) it reduced bacterial and mould counts by 10% and 1%, respectively (Velso *et al.*, 1974). Bacteria and mould levels were effectively reduced for approximately 3 weeks and there were no adverse effects on stock growth rates, feed efficiency or mortality. However, the potential risk of ingestion of the paraformaldehyde flakes by broilers (Seltzer *et al.*, 1969) and the toxicity of fumes to stock workers is a major problem (Reece *et al.*, 1979). Lime has been used to reduce microbial levels in litter by increasing the pH. Halbrook *et al.* (1951) reduced bacterial levels by 90% by adding lime to litter. However, increasing the pH of the litter or manure causes an un-desirable increase in ammonia emission (Bundy & Greene, 1995; Miner, 1995; Elliott & Collins, 1982) as a result of the shift in the ammonia / ammonium equilibrium in favour of ammonia (Section 4.2.1).

Strong oxidising agents such as potassium permanganate, hydrogen peroxide and chlorine not only oxidise ammonium (the most reduced form of nitrogen) to nitrate (the most oxidised form of nitrogen), but also destructively oxidise bacterial enzymes, thus having a disinfecting effect on the manure or litter (Elliott & Collins, 1983). Though these chemicals are effective at reducing ammonia loss from manure and litter, the addition renders the manure or litter unsuitable for land application due to increased chemical loading (Miner, 1995).

The addition of antibiotic growth promoters to fresh poultry manure significantly decreased the ammonia emission by approximately 50% (Kitai & Arakawa, 1979). A dietary addition of these antibiotics was also effective at decreasing ammonia emission from the fresh manure (Kitai & Arakawa, 1979). The use of antibiotic growth promoters in the poultry industry is restricted and marketing authorisation for remaining products is due to be withdrawn in 2006.

6.2.2 Acidification of Manure.

The acidification of manure and litter is effective in three ways; firstly it changes the pH of the substrate away from the microbial population's optimum level, hindering the decomposition process. Secondly, at an acidic pH any ammonia produced will be present as the non-volatile ammonium species and thirdly, the acids react with the ammonia / ammonium ion to form ammonium salts (Section 6.2.3).

A number of workers have attempted to utilise acids such as phosphoric acid (Moore *et al.*, 1996), lactic acid (Hörnig *et al.*, 1997), nitric acid (Kroodsma & Ogink, 1997) and volatile fatty acids (Parkhurst *et al.*, 1974), as well as acid-forming substances such as elemental sulphur (Mahimairaja *et al.*, 1994), ferric chloride (Boucher *et al.*, 1999), ferrous sulphate (Huff *et al.*, 1984), aluminium sulphate (Moore *et al.*, 1997) and calcium chloride (Kithome *et al.*, 1999) as litter or manure amendments.

The problems associated with this type of amendment are primarily related to the corrosive and toxic nature of acids, especially the corrosion of equipment (Hartung & Phillips, 1994), increased costs, safety problems (Vandre & Clemens, 1997) and the increased nutrient and or mineral content of the manure / litter. For example, the addition of phosphoric acid at 40g/kg of litter produced an 85% reduction in nitrogen loss compared to the control treatment, but also increased soluble phosphorus levels in the manure by 700% (Moore *et al.*, 1996). Also, the effects of inhalation or ingestion are also an issue where amendments are to be applied in stocked areas e.g. iron toxicity from ferrous sulphate or ferric chloride (Moore *et al.*, 1995). Due to the buffering capacity of the manure system, repeated applications of acids are required to maintain ammonia retention within the manure / litter substrate (Miner, 1995).

The reduction of microbial activity will reduce ammonia emissions and also slow or even prevents the natural decomposition process. As a result, there is no volume

reduction, which increases storage and handling costs, and no pathogen removal as there is no spontaneous heating caused by microbial activity, which makes the manure or litter less suited for land application (Miner, 1995).

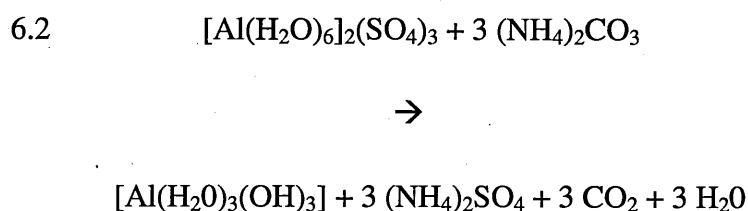
6.2.3 Binding of Ammonia / Ammonium.

The simplest type of chemical bonding of ammonia makes use of the hydrogen ion (H^+), forming the ammonium ion (NH_4^+). The equilibrium between ammonia and the ammonium ion can be maintained to favour the ammonium ion by maintaining a low pH (Elliott & Collins, 1983). The addition of acids to manure and litter (Section 6.2.1) are effective, as the acids reduce the pH and dissociate in the manure solution, preventing ammonia release by the formation of ammonium salts (Equation 6.1).



Similarly compounds such as superphosphate (monobasic calcium phosphate), ferrous sulphate and ferric chloride react with ammonia in a similar fashion. The addition of soluble calcium or magnesium salts cause precipitation of calcium or magnesium carbonate. This prevents the loss of carbon (as carbon dioxide), leaving an excess of hydrogen ions, decreasing the system pH and therefore increasing the retention of ammonium (Vandre & Clemens, 1997). Vandre & Clemens (1997) investigated the effectiveness of calcium, hydrogen and potassium ions (as chlorides) in reducing ammonia emissions. The calcium ion was found to be the most effective at reducing ammonia emissions, reducing pH and providing a buffering effect, by resisting pH increases which are associated with subsequent manure decomposition. The acidifying effect of the calcium ion is related to the precipitation of calcium carbonate (Fenn & Hossner, 1985) which is insoluble in water (McDuell, 1987). Witter & Kirchmann (1989b) also produced significant reductions in ammonia emission through the addition

of calcium and magnesium chloride to poultry manure. They concluded that the retention of nitrogen within the poultry manure was independent of the type of cation used. However, this is only likely to be the case for cations that produce carbonates that are insoluble in water. Potassium, which produces a water soluble carbonate, had no effect on either pH or nitrogen loss (Vandre & Clemens, 1997). A similar effect is seen with the type of anion used in the amendment. Sulphates are not as effective as chloride ions in reducing ammonia loss (Kithome *et al.*, 1999; Vandre & Clemens, 1997; Witter & Kirchmann, 1989b) because of their lower solubility (Fenn & Hossner, 1985). However, aluminium sulphate (alum) has been shown to be effective at reducing ammonia emission. This occurs not because of the anion, but as a result of the acidic properties of the cation, aluminium. The reaction of this hexaaqua metal with the ammonium carbonate present in the manure (as a result of urea hydrolysis) results in the formation of aluminium hydroxide and ammonium sulphate, with the evolution of carbon dioxide gas (Equation 6.2) as a result of acid formation (Kithome *et al.*, 1999; Moore *et al.*, 1997). This leaves the ammonium sulphate in an acidic aqueous solution, from which ammonia is not released.



Alum is more effective at reducing ammonia emissions than phosphoric acid, ferrous sulphate and ferric chloride (Moore *et al.*, 1996), providing the best ammonia retention and the lowest substrate pH. Alum has produced consistent reductions in ammonia losses (Moore *et al.*, 1995, 1997; Kithome *et al.*, 1999; Shreve *et al.*, 1995) and has had no negative effects on bird health when used on litter during production (Maurice *et al.*, 1998).

The direct combination of chemicals such as formaldehyde with ammonia / ammonium is also effective at reducing ammonia concentrations in the house (Seltzer *et al.*, 1969). While the combination of ammonia with glycoproteins present in the manure (as a result of dietary additions or un-digested feed) is also effective in preventing ammonia loss (Whyte, 1993). The addition of a source of metal ions such as iron and copper which are complex hexaaqua metals like aluminium, form stable amine complexes as a result of ligand exchange reactions (Elliott & Collins, 1983).

However, as with any amendment, an increase in manure / litter mineral content can be a major environmental problem e.g. aluminium from alum or phosphorus from phosphoric acid (Moore *et al.*, 1996). Also, the use of amendments within the housing system may provide application difficulties and there may be health concerns for stock and workmen depending on the characteristics of the amendment.

Once the ammonia is produced, it can also be bound onto an amendment by conversion to ammonium ions and subsequent exchange of these ammonium ions on the cation exchange sites within the substrate (Haynes & Sherlock, 1986). Some substrates like peat also adsorb ammonia (McCrory & Hobbs, 2001). The main advantage of this method of reducing ammonia emission is that it does not hinder the normal microbial decomposition of the manure (ensuring it is suitable for land application).

The direct application of clinoptilolite to manure reduced ammonia emissions by approximately 35-50% (Nakaue *et al.*, 1981; Koelliker *et al.*, 1978; Miner *et al.*, 1997). Though the addition of clinoptilolite to litter during production decreased the incidence of foot pad burns (Koelliker *et al.*, 1978), it was necessary to use a coarse clinoptilolite to combat the increased level of dust observed as a result of the fine clinoptilolite addition (Nakaue *et al.*, 1981).

In addition to zeolite, other substrates that have been tested as ammonia adsorbents include peat, clay and coir (mesocarp of the coconut fruit). These may be incorporated into the manure or litter, placed on the surface as a cover or placed in the air stream as a filter (Section 6.3). When peat was incorporated into poultry manure it produced a 25% reduction in ammonia emission (Mahimairaja *et al.*, 1994; Witter & Kirchmann, 1989a). Zeolites have given more variable results. Witter & Kirchmann (1989a) found only a 1.5% reduction in ammonia loss, whilst Airoidi *et al.* (1993) and Nakaue *et al.* (1981) found the ammonia loss reduction to be in the region of 35%.

As a surface cover, zeolite was considerably more effective at retaining ammonia, with emission reductions ranging from 44% (Kithome *et al.*, 1999) to 90% (Witter & Lopez-Real, 1988), while peat, clay and coir when used as covers reduced ammonia emissions by 25% (Barrington *et al.*, 1995), 60% (Witter & Lopez-Real, 1988) and 48% (Kithome *et al.*, 1999), respectively. Thus, adsorbents appear to be more effective as a cover than when they are incorporated into the manure (Witter & Lopez-Real, 1988).

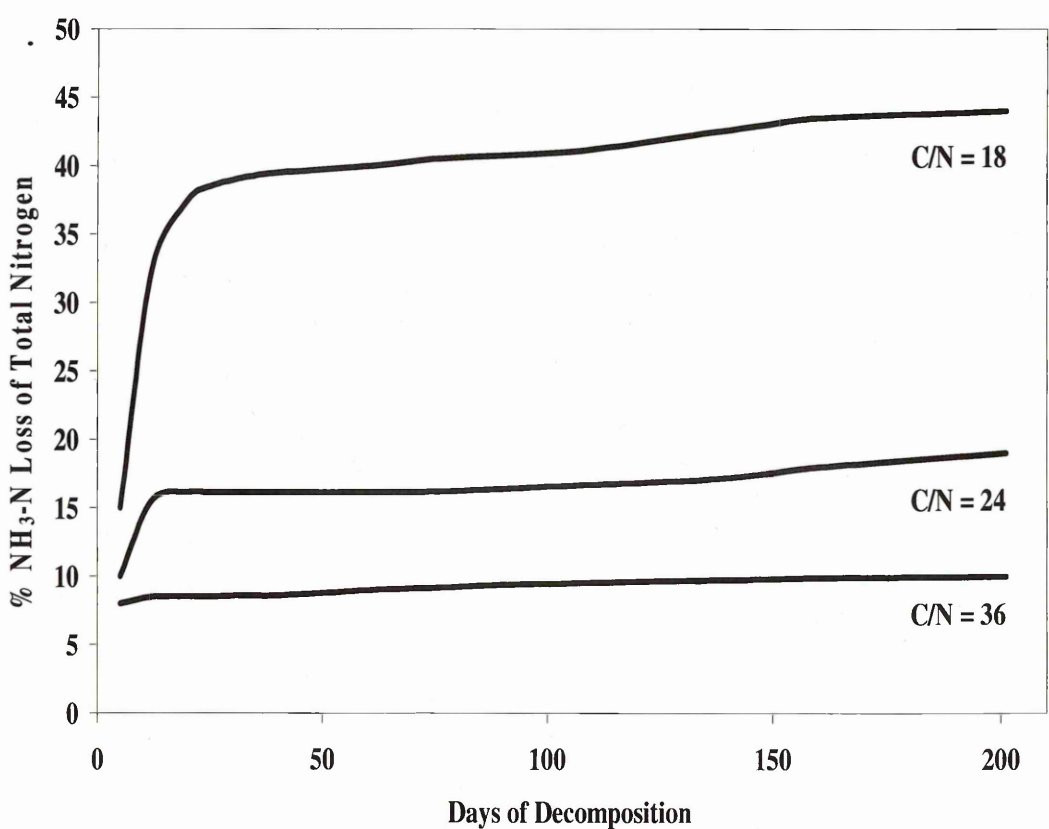
6.2.4 Carbon Based Amendments.

Substrates such as straw, sawdust, wood shavings or chips, paper waste and peat have been utilised as bedding materials in poultry management systems. These bedding materials have been added to poultry manure as part of manure composting operations during storage, to reduce ammonia emission and improve the composition of the end product. There are two main factors affecting the potential reduction of nitrogen loss in a manure composting system, these are the quantity of the carbon source added and the availability of the carbon source.

Adding a carbon substrate changes the C: N ratio of the manure, which is the primary factor influencing ammonia loss in composting or manure storage operations

(Switzenbaum *et al.*, 1994) (Section 3.8). The quantity of the carbon amendment added to the manure affects the C: N ratio. Kirchmann & Witter (1989) produced C: N ratios of 18, 24 & 36, and found that the amount of ammonia released decreased with increasing additions of straw (e.g. higher C: N ratio). At a C:N ratio of 36:1, only 9% of the initial nitrogen present was lost over the 200 days of the experiment, whereas lower C:N ratios released significantly more ammonia (Figure 6.1). Generally, C:N ratios in excess of 30:1 almost completely stop nitrogenous losses (Section 3.8). Surplus carbon is released as carbon dioxide, whilst nitrogen is retained in the biomass and re-cycled through successive generations (Golueke, 1992).

Figure 6.1 Cumulative ammonia loss from manure with increasing straw content during aerobic decomposition (Kirchmann & Witter, 1989).



The production of a stable, well matured poultry manure compost was shown to be possible by Mondini *et al.* (1996), who composted poultry manure with woodshavings (20 parts manure to 1 part woodshavings). This resulted in the formation of humic materials that were indicative of well matured composts. This process, however, resulted in a 44% loss of the initial nitrogen present. These nitrogen losses may be explained by the poor availability of the carbon source to the microbial population.

The effectiveness of carbonaceous amendments in reducing ammonia emission was affected by the availability of the carbon source to the microbial population (Mahimairaja *et al.*, 1994). Hence, an available energy source results in nitrogen retention by the temporary immobilisation of ammonium by the microbial population (Atkinson *et al.*, 1996).

The availability of a carbon source is affected by the resistance to hydrolysis of the carbon bonds within a carbohydrate (Miner *et al.*, 2000), with sugars being less resistant than starch, which is less resistant than cellulose and lignin (Begon *et al.*, 1990). The addition of these carbohydrates to poultry manure will produce good quality composts, but will result in ammonia loss as residual proteins in the manure are more easily decomposed than cellulose and lignin (Begon *et al.*, 1990). If more available carbohydrates were added, protein decomposition may be reduced.

The effect of adding simple sugars to decomposing manure largely depends on the aeration of the manure. In aerobic environments, sugars are metabolised into carbon dioxide by growing microbes, causing a decrease in pH. Under anaerobic conditions, sugars are broken down less efficiently resulting in the production of alcohols and organic acids which also lower the pH of the decomposing manure (Begon *et al.*, 1990). Therefore, by providing readily available sugars during decomposition, not only will nitrogen be retained in the microbial biomass, but also pH will reduce so favouring the

accumulation of produced ammonia as ammonium. This maintains the mineralised nitrogen in the manure solution where it can be incorporated into the biomass. This was demonstrated by Clemens *et al.* (2002) who added sucrose, glucose, sugar beet residues and organic household waste to dairy cow slurry, producing a decrease in slurry pH and a reduction in ammonia volatilisation.

The effectiveness of a carbonaceous amendment ultimately depends on the ability of the microbial biomass to degrade the carbon compounds (Witter & Lopez-Real, 1987). Once the carbon compounds are degraded they create a demand for nitrogen from the existing microbial population in order to sustain further microbial growth. This ideally occurs at a time when excess ammonia is being produced.

6.3 Extraction of Ammonia from the Air.

The extraction of ammonia from the air can be achieved by suspending amendments within the house air or by attaching filters or scrubbers to the exhaust vents. Substrates with a high affinity for ammonia (e.g. peat) would be more suited to placement in the air stream as a filter than substances such as zeolite, which has a high affinity for the ammonium ion and would thus be more effective when incorporated into or onto the surface of the manure. However, Witter & Kirchmann (1989a) found that placement of these substrates in the air as a filter was considerably more effective for both peat (59%) and zeolite (16%) compared to incorporation in the manure. Scrubbers typically trap gaseous pollutants by dissolving them in water (Peirce *et al.*, 1998). Scrubbers packed with an organic material such as peat, straw, zeolite or compost allow the addition of microbial or fungal cultures to be maintained within the scrubber, and the pollutant gases are degraded in the scrubber solution (McEldowney *et al.*, 1993). Chemicals such as sulphuric or phosphoric acid may be used to aid the removal of the ammonia gas

from the exhaust air (Hahne & Schuchardt, 1997; Weng *et al.*, 1984). This type of scrubber can produce a 95% reduction in ammonia levels in the exhaust air (Kroodsma *et al.*, 1996).

However, when the adsorbent is placed in the air stream as a filter, problems can arise from the accumulation of dust and feathers at the filters' surfaces, resulting in clogging (Koelliker *et al.*, 1978; Barrington *et al.*, 1995). The dust is not water soluble and an additional dust filter is required (Zeisig, 1988). The interaction between adsorbents and exhaust air is not always beneficial. A linear reduction in the effectiveness of zeolite in removing ammonia from the air occurred with increasing water adsorption, as a result of competition between water and ammonia molecules (Bernal *et al.*, 1993). In contrast a substrate such as peat requires moisture to improve adsorption (Norén, 1986) and when the ammonia removal efficiency drops, ammonia adsorption can be improved by washing the peat filters through with water (Scholtens *et al.*, 1988).

Chemical and water dependent scrubbers produce large quantities of waste that require both storage and disposal (Peirce *et al.*, 1998), and are expensive to install and operate (Peirce *et al.*, 1998; Barrington *et al.*, 1995). Biologically based scrubbers / filters are lower in cost yielding 70-80% ammonia removal efficiency (Scholtens *et al.*, 1988), but as with all scrubbing systems they are dependent on mechanical ventilation.

7. Experimental – The Effect of Temperature and Moisture Content during the Storage of Laying Hen Manure.

There was a need to quantify the effects of ambient temperature and moisture content on ammonia losses from freshly produced and stored laying hen manure. This information was required over short-term and long-term storage periods, and to examine the losses whilst the manure was still stored directly beneath the cages on manure belts or boards, and losses whilst the manure was stored away from the cage area. Short-term storage is used to describe the storage of manure in thin layers beneath the cage area, whilst long-term storage refers to the heaped manure stored following removal of the manure from the cage area. These data would allow changing management practices and housing types to be evaluated.

7.1 The Effect of Moisture Content and Ambient Temperature on the Gaseous Nitrogen Loss from Stored Laying Hen Manure.

7.1.1 Introduction.

The storage of freshly produced caged laying hen manure often occurs in close proximity to the stocked area. As manure is liable to ammonia volatilisation almost immediately following excretion (Chang & Flint, 1976) storage in this manner produces welfare and production problems. Excessive ammonia levels have a detrimental effect on the health and productivity of the stock (Charles & Payne, 1966a & b) and the health of the stock workers (Whyte, 1993). The temperature of the housing system is known to affect the rate of ammonia production within the manure and facilitate its loss from the manure. A second and equally important factor relating to ammonia production and loss from laying hen manure is the moisture content of the excreted manure (Section 4.4). This can be highly variable, but is strongly related to the mineral content of the feed (Smith *et al.*, 2000a). Though the reduction of these losses has been discussed by many authors (Section 4) there is still a need to quantify the effects of manure storage temperature and manure moisture content on nitrogenous losses from fresh caged laying hen manure during early storage.

The specific objectives of this study were to compare the effect of four storage temperatures and three laying hen manure moisture contents on the rate of nitrogen loss and manure decomposition characteristics over a 10-day storage period, in a replicated experimental design.

7.1.2 Materials & Methods.

A flock of 1200 ISA Brown laying hens were kept in cages within an environmentally controlled building from the start of their laying period until the commencement of the experimental feeding period. The birds were housed in six rows of cages (two blocks of cages each at three tier levels) stocked at 625cm²/bird. The housing system had separate belt cleaning of the manure for each block and tier, with no drying system. The powered ventilation used a negative pressure, side inlet, ridge extraction system.

A nutritionally complete laying hen ration (3 g/kg sodium) had been provided throughout lay (Table 7.1). At 78 weeks of age, one of three diets was provided to each individual tier of cages. The diets differed in their sodium concentrations (3, 6.4 & 10 g/kg). The three sodium concentrations were achieved by adding additional sodium chloride to the 3 g/kg sodium diet (previously fed to all the birds). This was intended to linearly increase the manure moisture content (Smith *et al.*, 2000a).

Manure was collected after the birds had been fed the experimental diets for at least two weeks. Each of the manure belts was cleaned and then manure produced over the next hour was collected. This was repeated three further times on that day and over four consecutive days. A period of high manure production (approximately 5 hours after the start of daylight) was chosen for the collection. The collected manure was immediately mixed and representative samples weighing approximately 1kg were transferred to individual galvanised metal trays to a depth of approximately 1cm. Two separate series of manure collections were made from the same flock of birds.

Table 7.1 Ingredient composition and calculated nutrient composition of the low sodium layer diet.

Ingredient	Amount (kg/tonne)
Ground wheat	500
Maize germ meal	200
Dehulled soya bean meal	133
Limestone	100
Full fat soya	45
Salt	2
Vitamin and mineral premix ¹	20
Calculated composition	
Metabolisable energy (MJ/kg)	11.6
Crude protein	154
Methionine plus cystine	7.3
Lysine	8.6
Calcium	42
Phosphorus	3.8
Potassium	6.3
Sodium	3.0

1. A proprietary vitamin and trace element supplement was used with a major nutrient composition of calcium (250g kg⁻¹), methionine (80g kg⁻¹), sodium (88g kg⁻¹), cupric sulphate (0.4g kg⁻¹), retinol (0.5mg kg⁻¹), tocopherol acetate (3.2mg kg⁻¹) and cholecalciferol (12ug kg⁻¹).

The experimental manure storage facility had eight environmentally-controlled chambers (22m³), with insulated concrete floors. Electric convector heaters and a positive pressure fan gave a constant air movement within the chamber, but varied the amount of outside air and re-circulated internal air to maintain the set temperature. The ventilation rate (%) and internal and external air temperature (°C) were measured continuously in each chamber, using data-logging equipment (Farmex, Reading, UK).

The manure samples were placed in one of the eight chambers and each chamber was kept at one of four constant temperatures (15, 19, 23 and 27°C). Four replicate trays of each of the 3 diets (twelve in total) were placed in each chamber (Figure 7.1) for a ten-

day period and representative samples were taken, using a random grid system from each tray after 0, 4, 8, 24, 48, 72, 144 and 240 hours (h) of storage. The first five time point samples were placed into sealed bags and rapidly frozen in liquid nitrogen and then stored at -20°C until analysed. The remaining samples were placed directly in a freezer (-20°C) until analysed. The second time replicate of the experimental protocol was conducted as described previously, but the allocated storage temperatures and positions of the trays from each manure moisture treatment within each chamber were re-randomised.

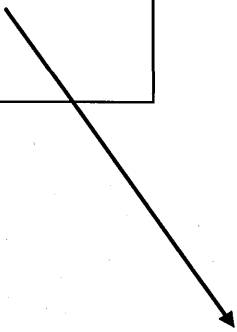
7.1.2.1 Laboratory Analysis.

Manure dry matter (A.O.A.C., 1990), Kjeldahl nitrogen content (Persson, 1996) and pH (Appendix 1) was determined from collected sub-samples from each time point.

Pooled samples of the un-stored manure from each of the three dietary treatments were freeze dried and ground to pass a 1mm sieve. The sodium contents of the three manure samples and the three diets were determined by atomic absorption spectrophotometry (Smith-Hieftje 1000, Thermo Jarrell Ash Corporation) after wet digestion (A.O.A.C., 1990).

Figure 7.1 Experimental Design:- The Effect of Moisture Content and Ambient Temperature on the Gaseous Nitrogen Loss from Stored Laying Hen Manure. First and (Second) time replicate.

Block 1	Block 2
15°C (15°C)	19°C (23°C)
23°C (23°C)	27°C (15°C)
19°C (27°C)	23°C (27°C)
27°C (19°C)	15°C (19°C)



Example Chamber Layout

Diet 2	Diet 1	Diet 2	Diet 2
Diet 1	Diet 3	Diet 1	Diet 3
Diet 3	Diet 1	Diet 2	Diet 3

7.1.2.2 Statistical Analysis.

The amounts of dry matter, nitrogen and water that remained in each of the individual sample trays at each of the sampling times were used to calculate the rates of loss during storage. Examination of these data indicated that the rates of loss were linear over the ten-day period, so linear regression analysis was used to determine the regression coefficient for each experimental unit. The rate of change of pH was non-linear and best fitted by an exponential curve function, in which an asymptote was reached after about 96h of storage. Exponential curves were fitted to these data from each experimental unit and the final asymptote was estimated.

The rates of loss of nitrogen and changes in the chemical characteristics of the manure samples were compared in a randomized block, split-plot analysis of variance that compared the effects of the four temperature treatments (main plots) and the three initial manure moisture contents (sub plots). The effects of temperature, manure moisture content and their interactions were partitioned into their linear and non-linear effects (Mead *et al.*, 1993).

7.1.3 Results.

The initial nitrogen contents of the three un-stored manure samples were 49, 51 and 50g nitrogen per kg of dry matter, and the initial pH levels were 7.0, 7.2 and 7.2, respectively. The determined sodium contents of the three diets were 3.2, 6.1 and 8.5 g/kg and the moisture contents of the three collected manure samples were 722 (SD = 4.6), 752 (SD = 4.1) and 796 (SD = 4.5) g/kg respectively. The determined sodium contents of the three un-stored manure samples were 5.4, 6.9 and 10.3 g/kg respectively. The mean storage temperatures achieved were 15.3, 18.5, 21.9 and 25.4°C. There were

no significant differences between the initial excreta compositions or the storage temperatures achieved between the two time replicates.

The rate of loss of dry matter, nitrogen and water increased linearly with increased ambient storage temperature ($P < 0.01$). The initial moisture content had a non-linear effect ($P < 0.001$) on the rates of loss of nitrogen and moisture. The greatest rate of nitrogen loss and lowest water loss occurred in the intermediate manure moisture treatment (Table 7.2). There was also a temperature times manure moisture interaction ($P < 0.05$), with a large increase in the rate of nitrogen loss from the intermediate manure moisture samples when they were stored at the highest temperature. The pH increased linearly ($P < 0.01$) with both increasing ambient storage temperature and increasing initial moisture content.

Table 7.2 The effect of four storage temperatures and three initial manure moisture contents on the rates of dry matter, nitrogen and water loss and the final pH from caged, laying hen manure over ten days of storage.

Rate of Dry Matter Loss (mg kg ⁻¹ h ⁻¹)	Ambient Storage Temperature °C	Initial Moisture Content g /kg			Storage Temperature P<0.001 Linear SEM = 6.79	Temperature x Moisture Interaction
		722	752	796		
	15.3	151.6	145.1	129.8	142.2	P>0.05
	18.5	176.0	160.0	158.2	164.8	SEM = 10.83
	21.9	194.7	178.1	162.9	178.6	
	25.4	202.1	194.2	178.0	191.4	
	Moisture Treatments P<0.01 Linear SEM =5.17	181.1	169.4	157.2		
Rate of Nitrogen Loss (mg kg ⁻¹ h ⁻¹)	Ambient Storage Temperature °C	Initial Moisture Content g /kg			Storage Temperature P<0.01 Linear SEM = 3.21	Temperature x Moisture Interaction
		722	752	796		
	15.3	32.7	40.2	34.5	35.8	P<0.05
	18.5	38.0	44.4	40.1	40.9	SEM = 4.86
	21.9	39.1	47.2	44.9	43.7	
	25.4	42.9	71.3	46.2	53.5	
	Moisture Treatments P<0.001 Non-Linear SEM =2.24	38.2	50.8	41.4		
Rate of Water Loss (mg kg ⁻¹ h ⁻¹)	Ambient Storage Temperature °C	Initial Moisture Content g /kg			Storage Temperature P<0.01 Linear SEM = 0.0995	Temperature x Moisture Interaction
		722	752	796		
	15.3	2.341	2.305	2.406	2.350	P>0.05
	18.5	2.697	2.483	2.549	2.576	SEM = 0.1394
	21.9	2.889	2.600	2.796	2.762	
	25.4	3.018	2.855	3.058	2.977	
	Moisture Treatments P<0.05 Non-Linear SEM =0.0598	2.736	2.561	2.702		
pH (asymptote)	Ambient Storage Temperature °C	Initial Moisture Content g /kg			Storage Temperature P<0.01 Linear SEM = 0.0917	Temperature x Moisture Interaction
		722	752	796		
	15.3	7.87	7.91	8.14	7.97	P>0.05
	18.5	8.19	8.21	8.28	8.23	SEM = 0.1116
	21.9	8.21	8.28	8.34	8.28	
	25.4	8.37	8.43	8.48	8.43	
	Moisture Treatments P<0.01 Linear SEM =0.0389	8.16	8.21	8.31		

7.1.4 Discussion.

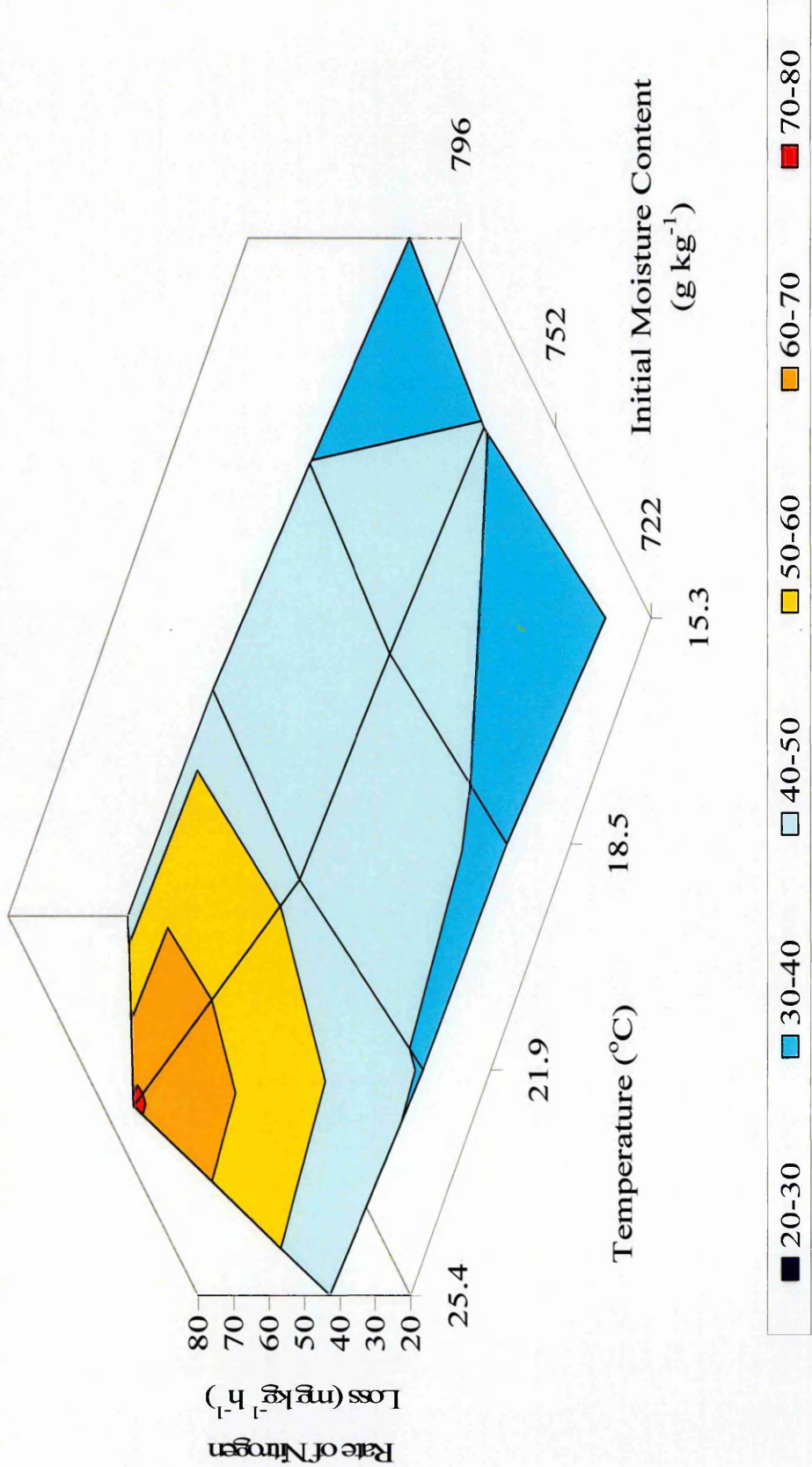
The data indicate that there was a substantial loss of gaseous material during short-term storage of the manure. Approximately 40g/kg of dry matter was lost over the ten-day storage period and one quarter of this total was gaseous nitrogen compounds. Carbon dioxide would comprise a large proportion of the remaining dry matter loss.

Increasing the ambient storage temperature increased the rate of loss of three major variable components (nitrogen, water and dry matter) in the manure, and gave an increase in the final pH. The rate of microbial activity increases with increasing temperatures (Groot Koerkamp, 1994) so it would be expected that gaseous losses of ammonia and carbon dioxide, the by-products of microbial metabolism, would be increased. The pH increased linearly ($P < 0.01$) with both increasing ambient storage temperature and increasing initial moisture content indicating an accumulation of ammonia (Fowler *et al.*, 1996).

Increasing the sodium content of the diets gave increases in the manure moisture contents that were comparable to those observed by Smith *et al.* (2000a). The increased sodium in the diets increased the sodium contents of the manure, but the sodium concentrations were much lower than the levels that would be expected to affect microbial growth (Jay, 1996).

The non-linear changes in nitrogen losses due to increasing manure moisture content were not expected (Figure 7.2). The intermediate moisture treatment had a faster rate of nitrogen loss ($P < 0.001$) compared to the other two treatments. The lack of an increase in the rate of nitrogen loss for the highest moisture treatment may have been due to an increase in anaerobic microbial decomposition.

Figure 7.2 The effect of storage temperature and initial moisture content on the rate of nitrogen loss during a 10 day storage period.



Schefferle (1965a) indicated that excessively wet litter restricts aerobic microbial proliferation. The high water content of the manure in the highest moisture treatment may have produced anaerobic conditions within the manure samples. Though anaerobic fermentation of manure produces gaseous nitrogen losses, the metabolic rate and proliferation of these microorganisms is much lower than would be expected with aerobic organisms (Groot Koerkamp, 1994). An increase of only 30 g/kg in the moisture content of the manure (lowest versus the intermediate moisture manure samples) gave a 33% increase in the rate of nitrogen loss. Nitrogen comprised 21% of the dry matter loss in the low moisture manure (722g/kg), whereas it increased to 30% of the dry matter loss in the intermediate moisture manure (752g/kg). High quantities of nitrogenous compounds are produced in aerobic microbial fermentations when there is a shortage of available carbohydrates (Higgins & Burns, 1975). As caged-laying hen manure is not incorporated with any litter material, undigested dietary residues are the only carbohydrates present. The amount of carbohydrate available to the microorganisms may have been limiting, so greater proportions of nitrogen-containing nutrients may have been used as energy sources when metabolism was increased by an increase in the available water.

7.1.5 Conclusions

This experiment demonstrated the effect that relatively small changes in excreta moisture content can have on gaseous nitrogen losses. Practical measures to reduce the moisture content of laying hen manure by reducing nutrient excesses in the diets could have a significant effect on the reduction of gaseous nitrogen losses to the environment from laying hen houses. The experiment has also shown that short-term storage of newly produced laying hen manure at high temperatures can significantly increase the loss of gaseous nitrogenous compounds into the environment. There was a 22%

increase in the rate of nitrogen loss from storage at 15.3°C to storage at 21.9°C. Many controlled environment commercial laying hen houses are kept in the region of 22°C, so this treatment is equivalent to the losses expected if the manure was kept within the laying house before long-term storage. The mean annual air temperature in central England is 9.5°C (Jones and Hulme, 1997). Although the experiment was conducted in the winter months, it was not possible to examine such low temperatures without avoiding diurnal temperature fluctuations. However, the lowest temperature examined in the present experiment indicates the reduction in ammonia losses that can be achieved by prompt removal of the manure from the house to a cooler storage area. One potential advantage of holding stored manure within the warmer layer house is that the increased airflow gives more rapid drying of the manure and reduction of its bulk. As the environmental variables that increase the evaporation of water from manure also increase the volatilisation of ammonia from the manure, this method of drying produces conflicts when attempting to reduce nitrogen loss from the manure (Groot Koerkamp *et al.*, 1998). Therefore, this faster rate of drying is also likely to produce increased nitrogenous losses from the manure.

7.2 The effect of Ambient Temperature on Losses of Volatile Nitrogen Compounds During the Long-Term Storage of Laying Hen Manure.

7.2.1 Introduction.

The long-term storage of laying hen manure occurs in a variety of ways (Section 5.1). Differing storage systems may maintain the stored manure at varying environmental temperatures and in varying quantities. There is a need to quantify the effect of temperature during the long-term storage of caged laying hen manure.

The specific objectives of this experiment were to compare the effect of four storage temperatures on 1 tonne heaps of laying hen manure and to quantify the rates of nitrogen loss over an 18-week storage period.

7.2.2 Materials & Methods

A single commercial flock of 60,000 caged laying hens were fed a nutritionally complete wheat and soya-bean meal based laying hen feed, and kept in a controlled environment house. Powered ventilation was by positive pressure, ridge inlet, side (of deep-pit) outlet. There were five tiers of cages stocked at 625cm²/bird. Manure was stored on integral collection belts (no drying system) and then a scraper system moved the manure to a pit that was directly below the cage area. Only manure that had been produced by the birds within 48 hours was collected, as it was scraped from the collection belts to the manure pit.

One tonne of manure was placed in each of the eight environmentally controlled storage chambers (22m³), as described in section 7.1.2. In addition to the existing environmental measurements, relative humidity controls were also available. Each

chamber was randomly allocated to one of four constant storage temperatures (12, 15, 20 and 25°C, two replicates of each temperature) and maintained between 70-75% relative humidity.

Representative manure samples were taken weekly using a soil corer for the first 4 weeks and every 2 weeks thereafter, up to 18 weeks. Samples were placed directly into a freezer (-20°C).

7.2.2.1 Laboratory Analysis.

Manure samples were analysed as described in Section 7.1.2.

The concentration of ammonia (ppm) was measured using a Dräger (Multiwarn II) Meter (Dräger Safety UK Ltd, Blyth, Northumberland. UK) in the exhaust air from each chamber and the ventilation rates were measured using a hand held anemometer (Airflow AV2 – TSI Incorporated, High Wycombe. UK). The ammonia concentration measured was converted to mg/m³ using standard gas equations (QUARG, 1993).

7.2.2.2 Statistical Analysis.

The amounts of dry matter, nitrogen and water that remained in each of the individual manure heaps at each of the sampling times were used to calculate the rates of loss during storage. Examination of these data indicated that the rates of loss were linear over the storage period, so linear regression analysis was used to determine the regression coefficient for each experimental unit. The rate of change of pH was non-linear and best fitted by an exponential curve function. Exponential curves were fitted to these data from each experimental unit and the final asymptote was estimated.

The rates of loss of nitrogen and changes in the chemical characteristics of the manure samples were compared in a randomized block analysis of variance that compared the

effects of the four temperature treatments. The effects of temperature were partitioned into their linear and non-linear effects (Mead *et al.*, 1993).

7.2.3 Results.

The mean measured storage temperatures actually achieved were 12.3°C, 15.3°C, 19.5°C and 25°C. There were only small differences between replicates ($\pm 1.25^\circ\text{C}$). The mean external temperature was 6.9°C during the experimental period. The relative humidity, in all the manure storage chambers, briefly rose above the set point in the first 10 days of the experimental period, due to the rapid moisture losses in this period. However, the mean relative humidity during the 18-week storage period was 73.8% (± 0.44).

The manure composition at the start of the experimental period was 303 g/kg (± 8.2) dry matter, 48 g (± 2.6) nitrogen per kg of dry matter and a pH of 7.8 (± 0.17).

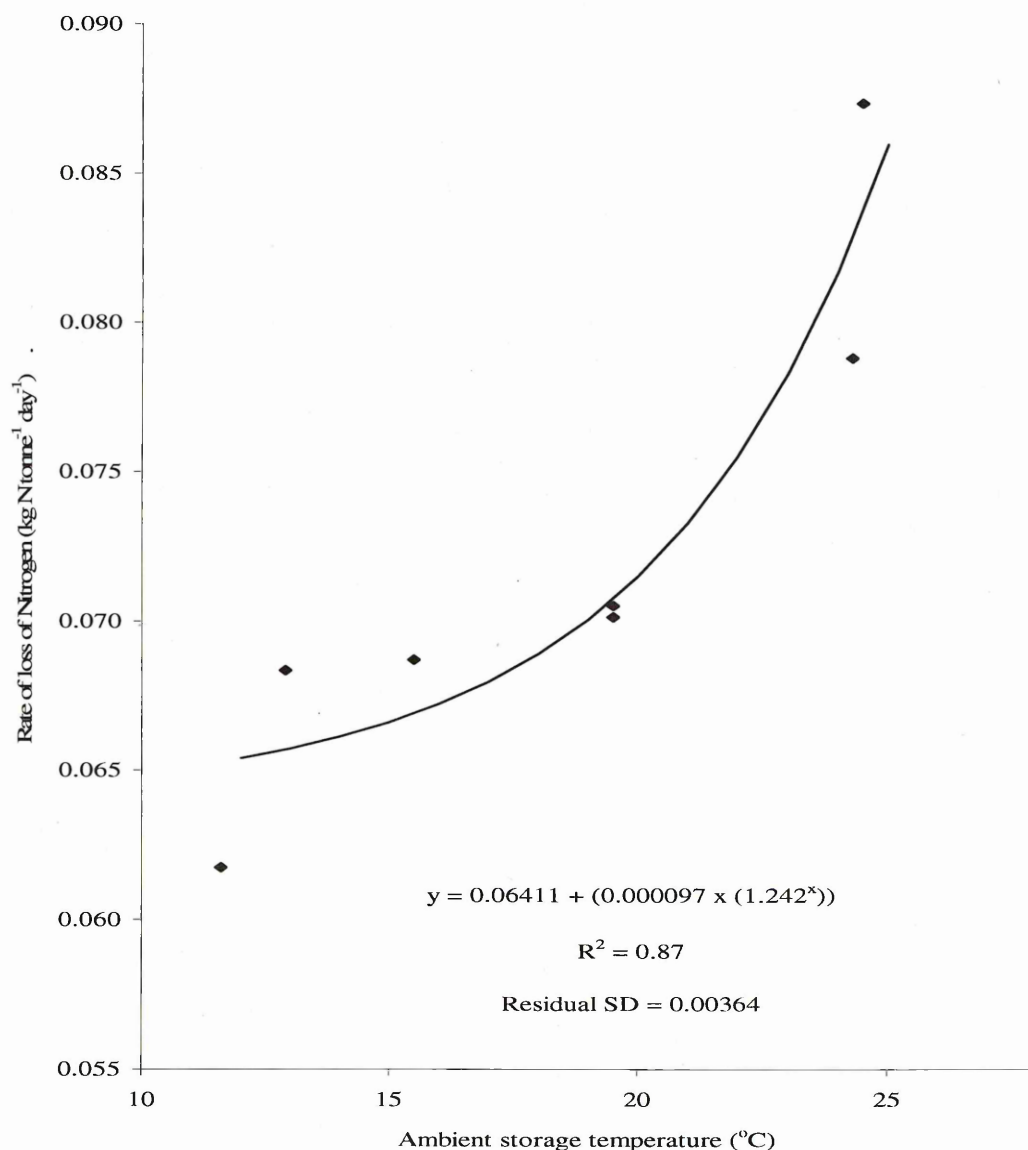
The weekly loss of volatile nitrogen compounds from the stored manure decreased linearly ($P < 0.001$) over the 18-week storage period (Table 7.3). Approximately 60% of the total nitrogen in the stored manure was lost over the 18-week storage period. The water content of the stored manure also decreased linearly ($P < 0.001$) over the 18-week storage period, with approximately 50% of the water initially present lost over the 18-week storage period. The stored manure had a rapid rise in pH, reaching a final constant level just below pH 9 ($P < 0.001$), after only approximately 14 days of storage.

Table 7.3 The effect of storage temperature on the rate of loss of the components of laying hen manure.

Storage Temperature	Total Volatile Nitrogen Loss	Nitrogen emitted as ammonia	Total Mass Loss	Water Loss	Dry Matter Loss	Final Constant pH
°C	kg Initial t ⁻¹ week ⁻¹	kg Initial t ⁻¹ week ⁻¹	kg Initial t ⁻¹ week ⁻¹	kg Initial t ⁻¹ week ⁻¹	kg Initial t ⁻¹ week ⁻¹	
12.3	0.455	0.126	25.69	18.67	8.46	8.48
15.3	0.481	0.249	24.66	18.03	8.19	8.41
19.5	0.492	0.271	28.15	20.43	8.67	8.41
24.4	0.582	0.313	29.95	22.80	8.90	8.31
SEM	0.0266	0.0135	0.823	0.412	0.849	0.0425
Significance of Difference	P<0.05 (Non-linear)	P<0.01 (Linear)	P<0.05 (Linear)	P=0.065 (Linear)	N.S. (P>0.05)	P=0.056 (Linear)

The rate of volatile nitrogen loss increased with increasing storage temperature. The increase was curvilinear ($P<0.05$) with a faster rate of loss occurring at temperatures greater than 20°C (Figure 7.3). The rate of loss of moisture in the present experiment increased linearly ($P<0.05$) with increasing temperatures, while there was no significant effect of temperature ($P>0.05$) on the dry matter loss. Increasing the ambient storage temperature tended ($P=0.056$) to give a lower final constant pH in the stored manure.

Figure 7.3 The effect of ambient storage temperature on the rate of loss of nitrogen from stored laying hen manure.



7.2.4 Discussion.

The results of the experiment indicate that there was a large loss of ammonia compounds from the stored manure and that ammonia formed the major part of this loss (Table 7.3). Approximately 60% of the total nitrogen in the stored manure was lost over the 18-week storage period.

The weekly loss of volatile nitrogen compounds from the stored manure decreased linearly over the 18-week storage period. This differs from other published data. Burnett & Dondero (1969) found an exponential decrease in the uric acid content of layer excreta over 3 weeks and a corresponding release of ammonia (Figure 3.3). Kirchmann & Witter (1989) also found an exponential release of ammonia from fresh poultry manure that included straw (Figure 6.1). The manure in the present experiment had a low carbohydrate content, because no bedding material was used in the production process. The manure was therefore relatively deficient in available energy for microbial growth. It is possible that a greater proportion of the relatively available nitrogen containing compounds in the manure were used as energy sources.

The much greater mass of stored manure studied in this experiment was another possible reason for the differences in the rate of nitrogen loss. Large masses of manure have relatively low surface area to volume ratio's, which would reduce moisture losses (Groot Koerkamp, 1994) and so a moisture content that was suitable for high microbial activity would have been maintained for a longer period. There was a linear rate of loss of nitrogen from the stored manure over the 18-weeks in the present experiment, but this rate of loss was unlikely to continue indefinitely because the nutrient loading and C:N ratio would have eventually become limiting (Kirchmann & Witter, 1989). However, the present experiment has demonstrated that, in practical situations, there is a continued loss of relatively large amounts of nitrogen from stored manure. Factors that affect the

rate of loss during this storage period could therefore be important to the release of volatile nitrogenous compounds into the environment.

The water content of the stored manure decreased linearly with approximately 50% of the water initially present being lost over the 18-week storage period. However, the water content as a proportion of the total mass remained relatively constant (at about 70%) because dry matter was being lost (as a result of microbial activity) at a similar rate during this storage period. Carr *et al.* (1990) concluded that ammonia loss from stored manure was only reduced when the water content was below 30%. The moisture content of stored manure in this experiment was within limits that were suitable for a high level of ammonia production throughout the storage period.

The stored manure had a rapid rise in pH, reaching a final constant level just below pH 9. This indicates that there was an initial accumulation of ammonia within the manure heaps. Carr *et al.* (1990) also found that the maximum pH was approximately 9. This pH is considered to be the optimum for the enzyme uricase, which is responsible for the initial aerobic breakdown of uric acid (Vogels & Van der Drift, 1976). The high pH in the stored manure would result in the majority of all nitrogen being lost as ammonia (Elliott & Collins, 1982, Fowler *et al.*, 1996). Thus, during the manure storage period favourable pH and moisture conditions enabled large amounts of aerobic nitrogen decomposition and therefore gaseous nitrogen loss.

The rate of volatile nitrogen loss increased with increasing storage temperature. The increase was curvilinear, with a faster rate of loss occurring at temperatures greater than 20°C (Figure 7.3). Groot Koerkamp (1994) also observed an increased decomposition rate of uric acid at temperatures above 20°C. The rate of loss of moisture in the present experiment increased linearly with increasing temperatures, while there was no significant effect of temperature on the dry matter loss. The relatively small differences

in moisture contents of the manure stored at the different temperatures were probably not large enough to affect the microbial decomposition of uric acid or limit the metabolism of aerobic organisms (Elliott & Collins, 1982).

Increasing the ambient storage temperature tended to give a lower final constant pH level in the stored manure. Ammonia is an alkaline substance, its production causes a localised increase in pH, shifting the ammonium : ammonia equilibrium in favour of ammonia (Fowler *et al.*, 1996). The pH of the manure solution is influenced by the ratio of ammonia and of carbon dioxide production. At higher storage temperatures where microbial activity is increased, ammonia may have been re-cycled into the microbial biomass (Atkinson *et al.*, 1996) and carbon dioxide production may then exceed ammonia production resulting in a reduced pH. A loss of ammonia also causes a reduction in pH (Vlek & Stumpe, 1978), so the lower pH at higher temperatures may indicate that a greater quantity of ammonia had been volatilised from the manure.

7.2.5 Conclusions

The data produced from this experiment have quantified the effect of temperature on the rate of gaseous nitrogen loss from 1 tonne heaps of stored laying hen manure. Increasing temperature increased total nitrogen loss and temperatures above 20°C produced the greater losses (18% increase when temperature was increased from 19.5°C to 24.4°C) than at temperatures below 20°C. Temperatures may often exceed 20°C in deep-pit egg production and manure storage systems. The storage of manure away from higher temperatures in a laying hen house, as achieved with belt-clean systems or in a stilt houses, would be likely to reduce the ambient temperatures and nitrogen losses from stored manure. However, the data show relatively small differences at temperatures below 20°C. Between 12.3 and 19.5°C there was only an 8% increase in

nitrogen loss. The average annual air temperature in the UK is 9.5°C (Jones & Hulme, 1997). If the manure from deep-pit systems was able to be stored at this average ambient environmental temperature (from 21°C to 9.5°C), then ammonia emissions would be reduced by approximately 12%, which is equivalent to 0.63 kt of ammonia, reducing the ammonia emission from the edible egg industry from 5.44 kt to 4.81 kt.

8. Experimental - The Effect of Carbohydrate Addition on Gaseous Nitrogen Loss from Stored Laying Hen Manure.

As storage temperature and manure moisture content can only be controlled to a limited extent, a further objective of this project was to develop a method of further reducing nitrogenous losses from stored laying hen manure. The literature review highlighted a number of techniques that have been used by other workers, which indicated variable results with many of the proposed methods. However, the addition of carbohydrates to laying hen manure, without the objective of compost production, had not been fully explored. The addition of carbohydrates to laying hen manure would increase the C: N ratio and potentially provide a non-toxic method of improving manure decomposition and promoting the retention of nitrogen.

Manure from caged laying hens does not have litter material incorporated, unlike in most other poultry production systems, so it is relatively deficient in available carbohydrate. If manure is deficient in available carbohydrates, microbes will utilise uric acid, proteins and other nitrogen-containing compounds as energy sources. Ammonia is a by-product of this process, so it is emitted from the stored manure (Higgins & Burns, 1975). Previously, the addition of carbohydrates to caged laying hen manure had been investigated largely in terms of producing stable poultry manure compost. Witter & Lopez-Real (1987) concluded that increasing the C: N ratio of animal manures by the addition of carbohydrates had given highly variable results, but overall there were some reductions in nitrogen loss. This reduction in ammonia emission probably occurred as a result of nitrogen being incorporated within the microbial biomass (Atkinson *et al.*, 1996). The availability of the carbon source to the

microbial population may determine the magnitude of the nitrogen retained in the biomass.

The objectives of this study were fourfold: firstly, to determine if adding carbohydrates to laying hen manure could give significant reductions in gaseous nitrogen losses during storage (Section 8.1); secondly, to examine whether the type of carbohydrate used gave differences in gaseous nitrogen loss (Sections 8.1 & 8.2); thirdly, to examine the level of addition of a selected carbohydrate on gaseous nitrogen loss (Section 8.3) and; finally; to establish the duration of the nitrogen retaining effects of the most effective level of the selected carbohydrate addition (Section 8.4).

8.1 The Effect of Carbohydrate Addition on Gaseous Nitrogen Loss from Stored Laying Hen Manure – Effects of Carbohydrate Availability.

8.1.1 Introduction.

The addition of a carbohydrate source to manure increases the C:N ratio of the manure. This has produced variable results as a nitrogen reduction technique (Witter & Lopez-Real, 1987) and is thought to reduce ammonia emissions from manure as a result of the incorporation of nitrogen containing compounds in the microbial biomass (Atkinson *et al.*, 1996). One reason for the variable results produced by using this technique for reducing nitrogenous losses from manure is the availability of the carbohydrate used to the microbial population. For example the availability of glucose (monosaccharide) > sucrose (disaccharide) > starch (polysaccharide) > cellulose (polysaccharide) (Begon *et al.*, 1990). This increasing resistance to microbial hydrolysis may reduce the effectiveness of the carbohydrate amendment in providing available carbon to facilitate the incorporation of nitrogenous material in the microbial biomass.

The objective of this experiment was to compare the effect of incorporating four carbohydrate sources (glucose, sucrose, starch and straw, at 8 g/kg fresh weight) on gaseous nitrogen losses from fresh laying hen manure.

8.1.2 Materials & Methods.

A single flock of 1,400 commercial caged laying hens were fed on a nutritionally-complete wheat and soya bean meal-based laying hen feed and kept in a controlled environment house, as described in section 7.1.2. The egesta of the birds were collected for approximately 20 hours on metal trays placed directly beneath the cages. The collected sample was mixed and 40 representative samples of 500g were taken. Each

sample was mixed and one of the four carbohydrates incorporated, and then placed on a plastic tray to an average depth of 1cm. No carbohydrates were added to the control treatment samples. Each treatment was replicated 8 times. The samples were stored in a single environmental chamber (314m³) for 7 days at 21°C (±2°C). This chamber did not have continuous relative humidity measurement or control, but good air movement characteristics gave no significant variation in humidity around the chamber.

Representative samples that included the whole vertical section of the stored sample were taken at the beginning and end of the experiment and stored at – 20°C. Complete vertical sections allowed the stored manure to be sampled evenly.

8.1.2.1 Laboratory Analysis.

Manure samples were analysed as in experiment 1 (Section 7.1.2). In addition, sub-samples were freeze dried and ground to pass through a 1mm sieve and analysed for electrical conductivity (Appendix 1), total carbon and sulphur (Leco SC-144DR, Leco Corporation, St Joseph, MI) and microbial Adenosine-Triphosphate (ATP) (Luminoskan TL Plus and Quantitative ATP Monitoring kit and ATP Releasing Agent, ThermoLabsystems, Vantaa, Finland) (Appendix 2). The microbial ATP content was used to calculate the total biomass assuming 1×10^{-15} g microbial ATP corresponds to a single bacterial cell (Lundin, 1999).

8.1.2.2 Statistical Analysis.

The changes in the measured variables were compared by a randomised block (position in chamber as blocking factor) analysis of variance, with the differences from the control treatment compared by a protected least significant difference test (Snedecor and Cochran, 1989). Any missing data values, in this or subsequent experiments were

estimated by an iterative approach using the method of Healy and Westamacott (1956) and the number of degrees of freedom reduced accordingly.

8.1.3 Results.

The manure composition at the start of the experimental period is described in Table 8.1.

Table 8.1 Initial manure composition (Experiment 8.1 – data expressed per initial kg of fresh manure).

Characteristic	Mean Composition (S.E.M.)
Dry matter (g/kg)	261.5 (2.90)
Nitrogen (g/kg)	13.6 (0.60)
Carbon (g/kg)	101.5 (1.00)
Sulphur (g/kg)	0.69 (0.015)
pH	6.12 (0.049)
Electrical conductivity (mS)	2.05 (0.028)
Bacterial numbers (no./g)	0.24×10^4 (0.005×10^4)

The addition of sucrose to the manure resulted in an increase in bacterial numbers ($P < 0.05$), a greater ($P < 0.05$) loss of carbon and a trend ($P > 0.05$) for a reduced loss of nitrogen compared to the control treatment (Table 8.2, Figure 8.1). Addition of starch to the manure gave similar effects to the sucrose addition, except that bacterial numbers only tended ($P > 0.05$) to be greater than the control and carbon loss was not ($P < 0.05$) different from the control. However, addition of glucose to the manure increased nitrogen loss and bacterial numbers ($P < 0.05$) and tended to increase carbon loss relative to the control. Addition of straw to the manure did not result in a change in bacterial numbers or rate of nitrogen loss compared to the control treatment ($P > 0.05$).

Table 8.2 The effect of carbohydrate additions on changes in the composition and characteristics of laying hen manure over 7 days of storage

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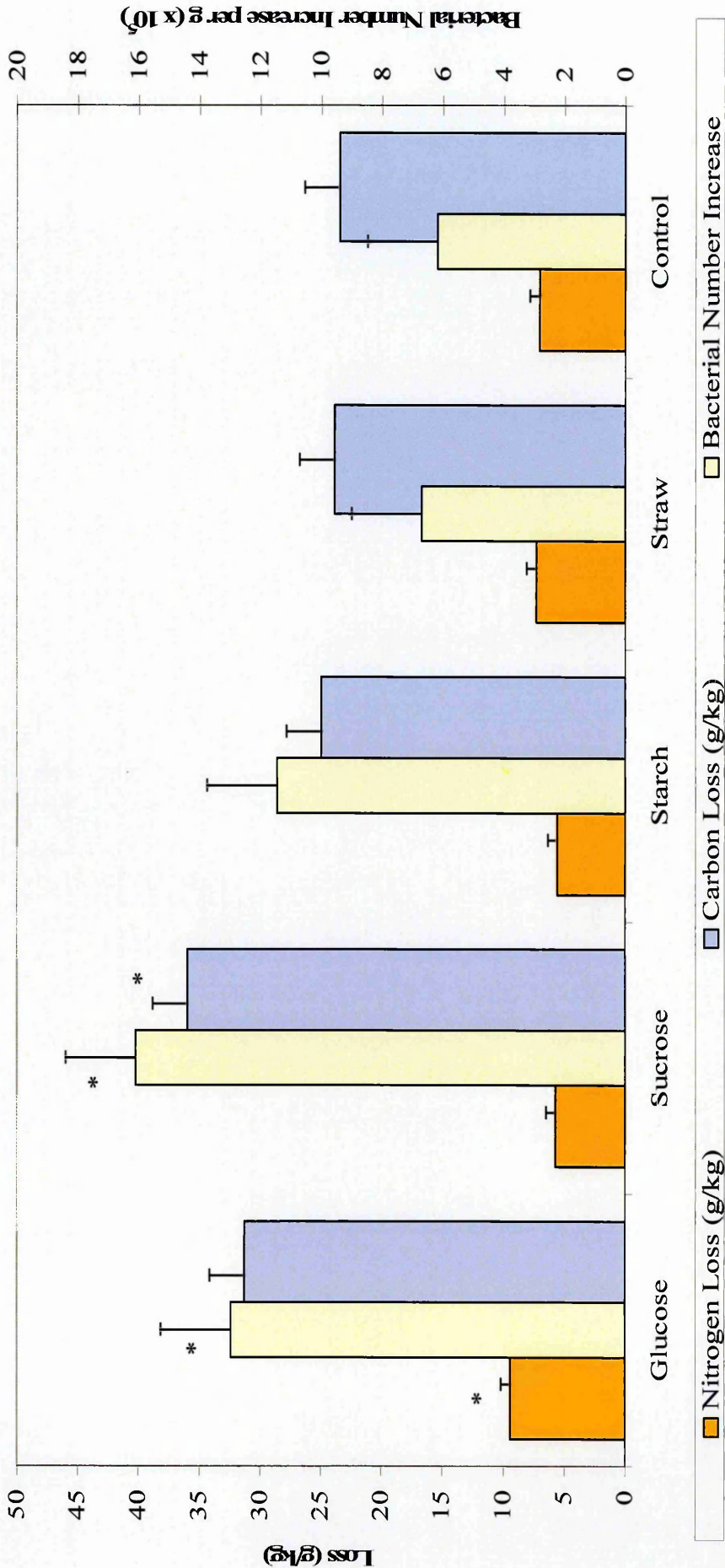
	Water loss (g/Initial kg)	Nitrogen loss (g/Initial kg) ¹	Carbon loss (g/Initial kg) ¹	Sulphur loss (g/Initial kg) ¹	pH Increase	E.C ² Increase (mS)	Bacterial number increase per g (x 10 ⁵)
Glucose	298*	9.42*	31.3	0.165	1.89*	0.10	12.95*
Sucrose	229	5.73	36.0*	0.150	2.23*	0.27	16.10*
Starch	216	5.58	25.0	0.113	1.83*	0.13	11.45
Straw	86	7.33	23.9	0.103	1.87*	0.04	6.7
Control	176	7.07	23.5	0.102	1.38	0.07	6.18
S.E.M.	35.8	0.776	2.85	0.0356	0.135	0.087	2.305
L.S.D.	103.8	2.289	8.39	0.1031	0.392	0.252	6.677
Statistical significance	P<0.01	P<0.05	P<0.05	N.S.	P<0.01	N.S.	P<0.05

¹Data expressed per initial kg of fresh manure.

²Electrical conductivity.

*Treatment mean significantly different from control treatment (P<0.05).

Figure 8.1 The effect of carbohydrate additions on changes in the composition of laying hen manure over 7 days of storage - I.



* = Treatment mean significantly different from control treatment (P<0.05).

Error bars represent the S.E.M.

8.1.4 Discussion.

The results indicate that some carbohydrate additions to laying hen manure can alter the loss of gaseous nitrogen from newly stored manure. These differences may be related to the growth of the bacterial population (Miner *et al.*, 2000) and how this synchronises with the availability of nitrogenous compounds within the manure. Nitrogen retention within stored manure is increased when microbial biomass formation is maximised (Atkinson *et al.*, 1996), but this formation depends not only on an available source of nitrogen, but also on a source of available energy being simultaneously available. Laying hen manure has a high nitrogen content but, because hens are fed highly digestible diets, it has a low available carbohydrate content. The addition of sucrose to the manure probably gave a suitably available source of energy for the microbes to use and so they were able to proliferate. Surprisingly, the addition of glucose gave a significant increase in nitrogen loss, microbial numbers were increased and there tended to be an increased carbon loss. It is possible that the glucose may have been metabolised too rapidly by the microbes and so this source of energy may not have synchronised with the ability of the microbes to incorporate nitrogen into biomass. Straw incorporation did not affect nitrogen loss or microbial numbers. Bacterial degradation of the high cellulose content of straw is slow (Begon *et al.*, 1990) and probably did not occur fast enough to release a significant amount of available energy within the short storage period. This was also found by Wilson & Hummel (1975) who ascribe the ineffectiveness of straw in dairy manure composting to be a result of its low availability.

8.1.5 Conclusions

The addition of sucrose and starch appears to have provided a suitably available carbohydrate source to laying hen manure, resulting in an increased bacterial population

within the manure during the first week of storage. Both, sucrose and starch tended ($P>0.05$) to reduce nitrogen losses. Nitrogen was probably incorporated into the population's biomass so reducing environmental loss. Glucose may have been too readily available to the bacterial population and as such the carbon and nitrogen availability did not coincide, resulting in a population boom and subsequent microbial utilisation of nitrogenous compounds for their energy requirements, releasing the excess nitrogen as ammonia. Straw may have been too resistant to breakdown and did not provide a readily available carbon source to the microbial population during this short time scale. Thus, these two amendments were un-suitable for retaining nitrogen in short-term storage.

8.2 The Effect of Carbohydrate Addition on Gaseous Nitrogen Loss from Stored Laying Hen Manure – II.

8.2.1 Introduction.

The previous experiment “The Effect of Carbohydrate Addition on Gaseous Nitrogen Loss from Stored Laying Hen Manure – Effects of Carbohydrate Availability.” (Section 8.1) highlighted the potential of sucrose and starch as possible amendments to aid the reduction of nitrogen losses from decomposing caged laying hen manure.

The objective of this experiment was to quantify the nitrogen loss from stored laying hen manure when one of three carbohydrate sources (sucrose, maltose and starch plus α -amylase (E.C. Number 3.2.1.1.) (1.6g in 80ml distilled water, 1.16×10^3 activity units per ml) were incorporated at 8g/kg. Maltose was selected in addition to the carbohydrates identified by the previous experiment as a second disaccharide and because it is a major breakdown product of starch hydrolysis. The starch plus α -amylase treatment was selected instead of starch alone as it is a less expensive means of providing a source of disaccharides. A fourth control treatment (no carbohydrate addition) was also included in the experiment.

8.2.2 Materials & Methods.

A single commercial flock of 60,000 caged laying hens were fed a nutritionally complete wheat and soya-bean meal based laying hen feed, and kept in a controlled environment house. The system used a scraper to move manure to a pit below the cage area. There was no manure drying system. There were five tiers of cages stocked at 625cm²/bird. Powered ventilation was by positive pressure, ridge inlet, side (of deep-pit) outlet.

Approximately 100kg of freshly produced egesta were collected on metal trays placed on the integral collection boards from the birds over 2 days. The manure was mixed and 2.0kg aliquots were weighed, mixed with one of the three carbohydrates and then placed on a plastic lined, metal tray to a depth of approximately 2.5cm. The starch and α -amylase treatment involved adding 40ml of additional water per kg of manure and this decreased the dry matter content of the starting manure sample. Starch and the α -amylase solution were only combined when mixed into the manure. No carbohydrates were added to the control treatment samples. Each treatment was replicated 12 times. The samples were stored for 7 days in one of four environmentally controlled storage chambers (as described in section 7.2.2), that were all maintained at 21°C ($\pm 1^\circ\text{C}$) and a relative humidity of 73% ($\pm 2\%$). Ventilation rate, relative humidity and the ambient internal and external air temperatures were continuously monitored with data-logging equipment (Farmex, Reading, UK). Four storage chambers were used in order that the large number of samples could all be kept at the same temperature and with the same air movement patterns.

Representative samples that included the whole vertical section of the stored sample were taken at the beginning and end of the experiment and freeze-dried.

8.2.2.1 Laboratory Analysis.

Laboratory analyses were as described in Section 8.1.2, except that total nitrogen was measured (Leco FP-528, Leco Corporation, St Joseph, MI) instead of Kjeldahl nitrogen.

The experiments reported in chapters 8.2, 8.3 and 8.4 used a different nitrogen analysis technique than the experiments reported in chapters 7.1, 7.2 and 8.1. Samples from the experiments reported in chapters 8.2 were analysed using both techniques and there was no significant difference in the determined results. The Leco nitrogen analyser

measures total nitrogen by a combustion method, whilst Kjeldahl nitrogen is a measure of organically bound nitrogen and ammonium. Kjeldahl nitrogen does not measure nitrate or nitrite, but these are not normally present in fresh manure (Kirchmann & Witter, 1989; Groot Koerkamp, 1994).

In addition, water activity was determined using a chilled mirror, dewpoint system (Decagon, Aqualab series 3 supplied by Labcell Ltd, Alton, Hampshire, UK) from the reserve samples that had been previously frozen (-20°C) since collection.

8.2.2.2 Statistical Analysis.

The changes in the measured variables were compared by a randomised block (chamber as blocking factor) analysis of variance, with the differences from the control treatment compared by a protected least significant difference test (Snedecor and Cochran, 1989).

8.2.3 Results.

The manure composition at the start of the experimental period is described in Table 8.3

Table 8.3 Initial manure composition (Experiment 8.2 – data expressed per initial kg of fresh manure).

Characteristic	Mean Composition (S.E.M.)
Dry matter (g/kg)	302.3 (3.90)
Nitrogen (g/kg)	17.9 (0.30)
Carbon (g/kg)	104.6 (0.97)
Sulphur (g/kg)	0.51 (0.015)
pH	6.88 (0.032)
Electrical conductivity (mS)	2.59 (0.024)
Bacterial numbers (no./g)	0.84×10^4 (0.067×10^4)
Water Activity	0.978 (0.0006)

The results (Table 8.4) showed that there was an increase ($P < 0.05$) in bacterial numbers and a lower nitrogen loss ($P < 0.05$) when either sucrose or maltose was mixed with stored laying hen manure, but only sucrose gave a significant pH difference ($P < 0.05$). The starch plus α -amylase treatment did not give any differences ($P > 0.05$) in the storage properties or nitrogen losses from the manure.

Table 8.4 The effect of carbohydrate additions on changes in the composition and characteristics of laying hen manure over 7 days of storage
- II.

	Water Loss (g/Initial kg)	Nitrogen Loss ¹ (g/Initial kg)	Carbon Loss ¹ (g/Initial kg)	Sulphur Loss ¹⁺² (g/Initial kg)	pH increase	E.C. Increase (mS)	Bacterial Number Increase per g(x 10 ⁴)	Water Activity Decrease (x 10 ⁻⁵) ³
Sucrose	155	3.73*	17.5	0.074	0.91*	0.174	10.32*	5.9
Maltose	154	3.85*	15.3	-0.009	1.06	0.266	10.37*	5.1
Starch & α-amylase	143	7.05	18.6	0.092	1.28	0.178	4.99	0.9
Control	141	6.65	19.6	0.056	1.31	0.064	3.79	2.6
S.E.M.	9.0	0.738	1.83	0.0335	0.107	0.0763	1.361	1.29
L.S.D.	25.6	2.109	5.242	0.0958	0.306	0.2192	3.886	3.68
Statistical Significance	N.S.	P<0.01	N.S.	N.S.	P<0.05	N.S.	P<0.01	P<0.05

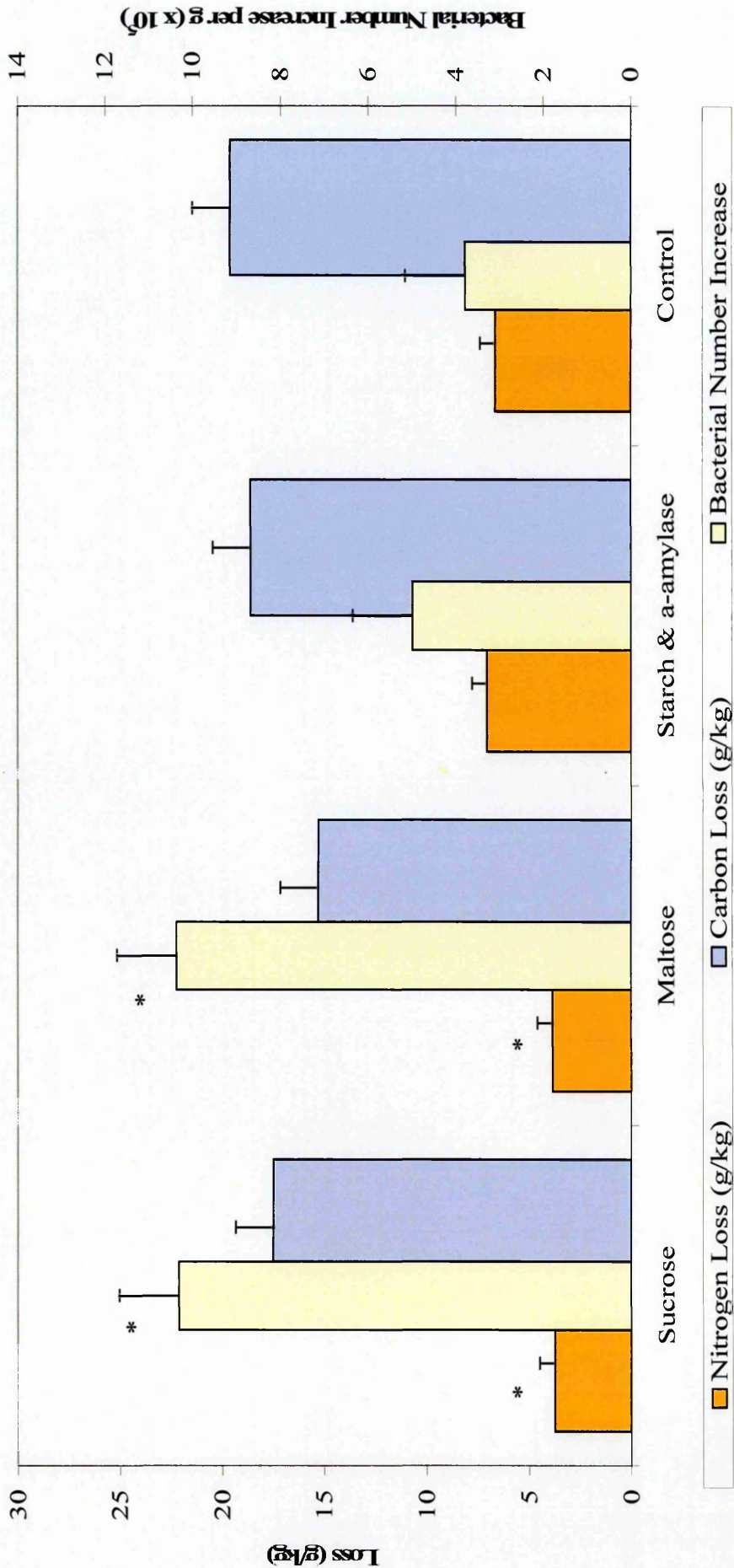
¹ Data expressed per initial kg of fresh manure.

² Negative value represents a measured increase in the variable.

³ Water Activity (A_w) = Equilibrium Relative Humidity / 100.

* Treatment mean significantly different from control treatment (P<0.05).

Figure 8.2 The effect of carbohydrate additions on changes in the composition of laying hen manure stored over 7 days - II.



* = Treatment mean significantly different from control treatment (P<0.05).

Error bars represent the S.E.M.

8.2.4 Discussion.

The significant increase in the bacterial population in the sucrose and maltose amended manure, coupled with the reduction in nitrogen loss in these samples, indicates that the additional carbon supplied allowed the growth of the bacterial population and the incorporation of nitrogenous material into the biomass. The availability of the disaccharides to the microbial population appeared to coincide with the breakdown of the uric acid to ammonium carbonate, the stage at which it is available to the microbial population and thus can be incorporated into the microbial biomass.

The pH level of all the treatments decreased compared to the control, though only the sucrose amendment produced significant reduction. This reduction in pH occurs as a result of the breakdown of the carbohydrate which results in aqueous, acidic compounds (Sommer & Sherlock, 1996).

The starch plus α -amylase treatment did not produce any differences in the storage properties or nitrogen losses from the manure, which may have been as a result of the additional water supplied when adding the enzyme. Reece *et al.* (1979) and Parkhurst *et al.* (1974) examined manure additives and attributed increased nitrogen losses in some treatments to them being in aqueous form.

8.2.5 Conclusions

Sucrose and maltose provided sufficient available carbohydrate to support a significant growth in the microbial population. This gave a significant reduction in nitrogen loss from the stored manure, most probably due to the nitrogen being incorporated into the microbial biomass. The addition of an amendment to stored manure would increase storage and handling costs due to the increased volume, and this would be in addition to

the cost of the additive itself, thus it is necessary to determine at what level any amendment is effective. As sucrose is available at a lower cost than maltose, it was used to determine the optimum level of amendment.

8.3 The Effect of Different Levels of Sucrose on Gaseous Nitrogen Loss from Stored Laying Hen Manure.

8.3.1 Introduction.

Sections 8.1 and 8.2 have established the effectiveness of disaccharides in reducing nitrogen losses during the storage of caged laying hen manure. This had been tested at 8g/kg. Increasing the application rate may increase the amount of nitrogen retained in the microbial biomass.

The objective of this experiment was to determine the effect on nitrogen loss from stored laying hen manure when sucrose was incorporated at 0, 5, 10, 20, 35 and 50g/kg.

8.3.2 Materials & Methods.

A single flock of 1,400 caged laying hens were fed a nutritionally complete wheat and soya-bean meal based laying hen feed, and kept in a controlled environment house, as described in section 7.1.2 . Approximately 100kg of freshly produced manure was collected directly on the collection belt over 2 days. The manure was mixed and then the different levels of sucrose were added to replicate 2.0kg manure samples. The samples were placed on plastic lined metal trays and stored in one of four environmental chambers (as described in section 7.2.2) for 7 days at 21°C ($\pm 1^\circ\text{C}$) and a relative humidity of 73% ($\pm 2\%$). Representative samples were taken at the beginning and end of the experiment and freeze-dried.

8.3.2.1 Laboratory Analysis.

Laboratory analyses are as described in section 8.2.2.

8.3.2.2 Statistical Analysis.

The changes in the measured variables were compared using a randomised block (chamber as blocking factor) analysis of variance, with the treatment effects partitioned into their linear and non-linear effects (Mead *et al.*, 1993).

8.3.3 Results.

The manure composition at the start of the experimental period is described in Table 8.5.

Table 8.5 Initial manure composition (Experiment 8.3 – data expressed per initial kg of fresh manure).

Characteristic	Mean Composition (S.E.M.)
Dry matter (g/kg)	290.4 (2.30)
Nitrogen (g/kg)	11.99 (0.225)
Carbon (g/kg)	106.6 (1.20)
Sulphur (g/kg)	0.63 (0.018)
pH	7.10 (0.037)
Electrical conductivity (mS)	1.93 (0.015)
Bacterial numbers (no./g)	1.19×10^4 (0.103×10^4)
Water Activity	0.974 (0.0008)

The increasing levels of sucrose addition gave a non-linear reduction in nitrogen loss ($P < 0.01$ - Table 8.6). The loss of nitrogen was correlated with the increase in the bacterial population which was also non-linear ($P < 0.05$), with bacterial populations increasing as nitrogen loss decreased. The maximum increase in bacterial numbers was obtained at 20g/kg of added sucrose (Figure 8.3), but this did not correlate with the lowest level of nitrogen loss (35g/kg).

Table 8.6 The effect of level of added sucrose on changes in the composition and characteristics of laying hen manure over 7 days storage.

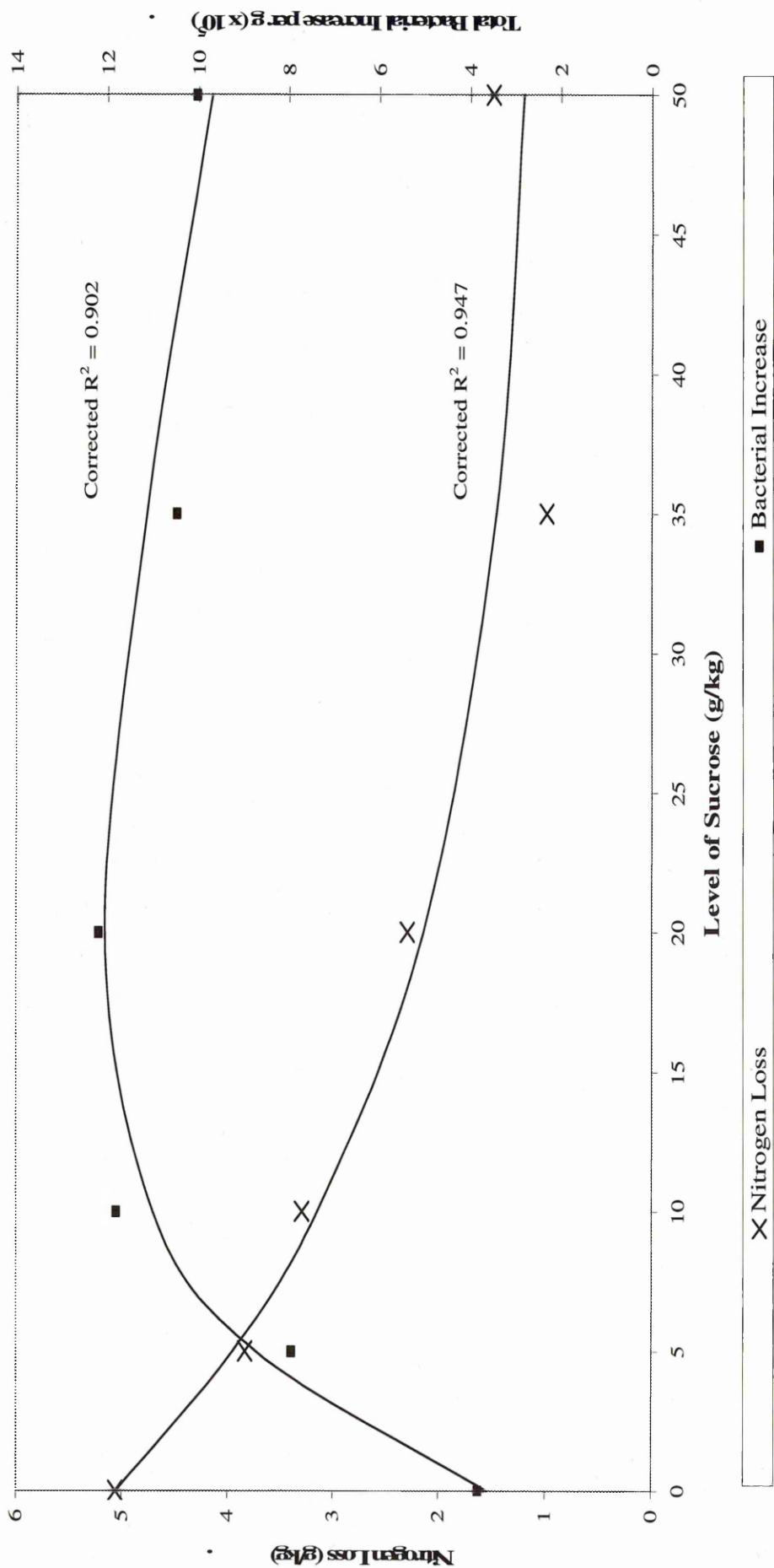
	Water Loss	Nitrogen Loss	Carbon Loss ¹	Sulphur Loss ¹	pH Increase ²	E.C. Increase ²	Total Bacterial Increase	Water Activity Decrease
Level of Sucrose (g/kg)	(g/Initial kg)	(g/Initial kg)	(g/Initial kg)	(g/Initial kg)		(mS)	per g (x 10 ⁴)	(x 10 ⁻³) ³
0	144	5.06	19.6	0.154	1.066	-0.007	3.80	8.5
5	151	3.83	15.3	0.063	0.920	-0.064	7.90	6.1
10	172	3.29	16.3	0.008	0.697	0.002	11.78	1.9
20	169	2.30	16.8	0.005	0.340	0.095	12.17	3.4
35	144	0.99	13.8	0.031	-0.138	0.331	10.46	0.9
50	136	1.50	16.6	0.021	-0.208	0.423	10.04	-2.1
S.E.M.	14.9	0.523	3.20	0.0485	0.1523	0.0637	1.910	2.49
Statistical Significance								
Linear	N.S.	P<0.001	N.S.	N.S.	P<0.001	P<0.001	N.S.	P<0.01
Quadratic	N.S.	P<0.01	N.S.	N.S.	N.S.	N.S.	P<0.05	N.S.
Cubic	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Deviations	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

¹ Data expressed per initial kg of fresh manure.

² Negative value represents a decrease in the variable.

³ Water Activity (A_w) = Equilibrium relative humidity / 100

Figure 8.3 The loss of nitrogen and the increase of the bacterial population in laying hen manure stored for 7 days, with increasing amounts of added sucrose.



Increasing levels of sucrose gave linear changes in the rate of pH change ($P < 0.001$). High levels of sucrose addition ($> 35\text{g/kg}$) produced a decrease in the pH level. Also, a decrease in water activity was observed from the stored manure samples. The magnitude of this decrease was reduced with increasing sucrose addition ($P < 0.01$).

8.3.4 Discussion.

Increasing the level of the sucrose amendment resulted in further reductions in nitrogen losses from the stored manure. This was also seen by Winsor & Pollard (1956) & Okereke & Meints (1985). This reduction in nitrogen losses may be explained by the incremental reduction in pH levels with increasing levels of sucrose inclusion. Lower pH levels would have resulted in any ammonia present in the manure to be present in the form of ammonium, as a result of the effect of pH on the ammonia : ammonium equilibrium (Section 4.2.1). The level of increase in the bacterial population with increased levels of sucrose addition may also have been reduced by this lower pH. The low pH may have inhibited the growth of the microbial population and may hence have reduced the production of ammonia.

A decrease in water activity was observed from the stored manure samples. The magnitude of this decrease was reduced with increasing sucrose addition. This may have been due to the pH decrease and the release of water during acid formation resulting from the breakdown of the sucrose (Sommer & Sherlock, 1996).

8.3.5 Conclusions

Increasing levels of sucrose addition to stored laying hen manure reduced nitrogen losses and increased bacterial populations. Even a small addition (5g/kg) reduced nitrogen losses by 25% and more than doubled the bacterial population. The largest nitrogen loss (80% reduction) was obtained by the addition of 35g/kg of sucrose.

However, there was not a linear dose response effect on nitrogen retention with increasing sucrose addition. The nitrogen retention levelled out around 35 g/kg and subsequent sucrose addition provided no further reduction in nitrogen loss.

8.4 The Effect of Sucrose Amendment on Gaseous Nitrogen Loss from Laying Hen Manure over Time.

8.4.1 Introduction.

The previous experiment “The Effect of Different Levels of Sucrose on Gaseous Nitrogen Loss from Stored Laying Hen Manure.” (Section 8.3) produced significant reductions in the level of nitrogen loss from caged laying hen manure as a result of the addition of sucrose. The highest reduction of nitrogen loss was achieved at an inclusion rate of 35g/kg. Though the success of this amendment over a 7-day storage period is known, the duration of the nitrogen retention is uncertain. The longevity of the nitrogen retaining effects of the sucrose amendment is an important factor in determining its usefulness as a commercial amendment that promotes nitrogen retention in laying hen manure.

The objective of this experiment was to establish the duration of the nitrogen retaining effects of sucrose when added to laying hen manure at 35g/kg during a 12 week storage period.

8.4.2 Materials & Methods.

A single flock of 1,400 commercial caged laying hens were fed a nutritionally complete wheat and soya-bean meal based feed, and kept in a controlled environment house (as described in section 7.1.2). Approximately 350kg of manure produced over a 48-hour period was collected. 10kg of sub-samples of manure were mixed in a cement mixer and sucrose was added at 35g/kg to the treated samples. Control samples were mixed in the same manner as the amended samples to ensure uniformity of aeration. The samples were placed in metal trays and stored in one of two environmental chambers (as described in section 7.2.2) at 21°C ($\pm 1^\circ\text{C}$) for 12 weeks. Representative sub-samples

that included the whole vertical section of the stored sample were taken after 0, 1, 2, 4, 8 and 12 weeks of storage. The samples were then freeze-dried before storage.

8.4.2.1 Laboratory Analysis.

Samples were analysed as described in section 8.2.2.

8.4.2.2 Statistical Analysis.

The characteristics of the amended and the control manure samples at each time point were compared by a randomised block (positional block as blocking factor) analysis of variance (Snedecor & Cochran, 1989). The time times treatment interaction was determined using a repeated measures, randomised block (positional block as blocking factor) analysis of variance (Snedecor & Cochran, 1989).

8.4.3 Results.

The initial manure composition was similar to the previously reported experiments (Table 8.7).

The addition of sucrose to manure over the 12-week storage period had no effect on the magnitude of the overall nitrogen loss, with both treated and un-treated manure losing approximately 70% of the initial nitrogen present (Table 8.8). However, there was an interaction between the addition of the sucrose and the length of storage ($P < 0.001$), where the nitrogen loss was significantly slower from sucrose amended manure until week 4 (Figure 8.4). The addition of sucrose to the manure increased bacterial numbers after two weeks ($P < 0.001$ - Table 8.8) and there was an interaction between the amendment and the storage time ($P < 0.01$), where the increase in bacterial numbers significantly increased in sucrose amended manure after week 2 (Figure 8.5).

Table 8.7 The Characteristics of the Laying Hen Manure at the start of the experimental period

Characteristic	With Sucrose	Control	Significance of Difference	L.S.D.
Dry matter (g/kg)	295.4	268.4	P<0.001	7.68
S.E.M.	1.86	3.27		
Nitrogen (g/kg)	10.07	9.96	N.S	1.04
S.E.M.	0.362	0.355		
Carbon (g/kg)	115.02	103.69	P<0.001	4.20
S.E.M.	0.995	1.80		
Sulphur (g/kg)	0.52	0.51	N.S.	0.03
S.E.M.	0.009	0.009		
pH	7.6	7.5	N.S.	0.12
S.E.M.	0.04	0.05		
Electrical conductivity (mS)	1.66	1.81	P<0.001	0.05
S.E.M.	0.018	0.016		
Bacterial numbers (no./g)	0.89 x 10 ⁴	0.89 x 10 ⁴	N.S.	0.33 x 10 ⁴
S.E.M.	0.067 x 10 ⁴	0.15 x 10 ⁴		

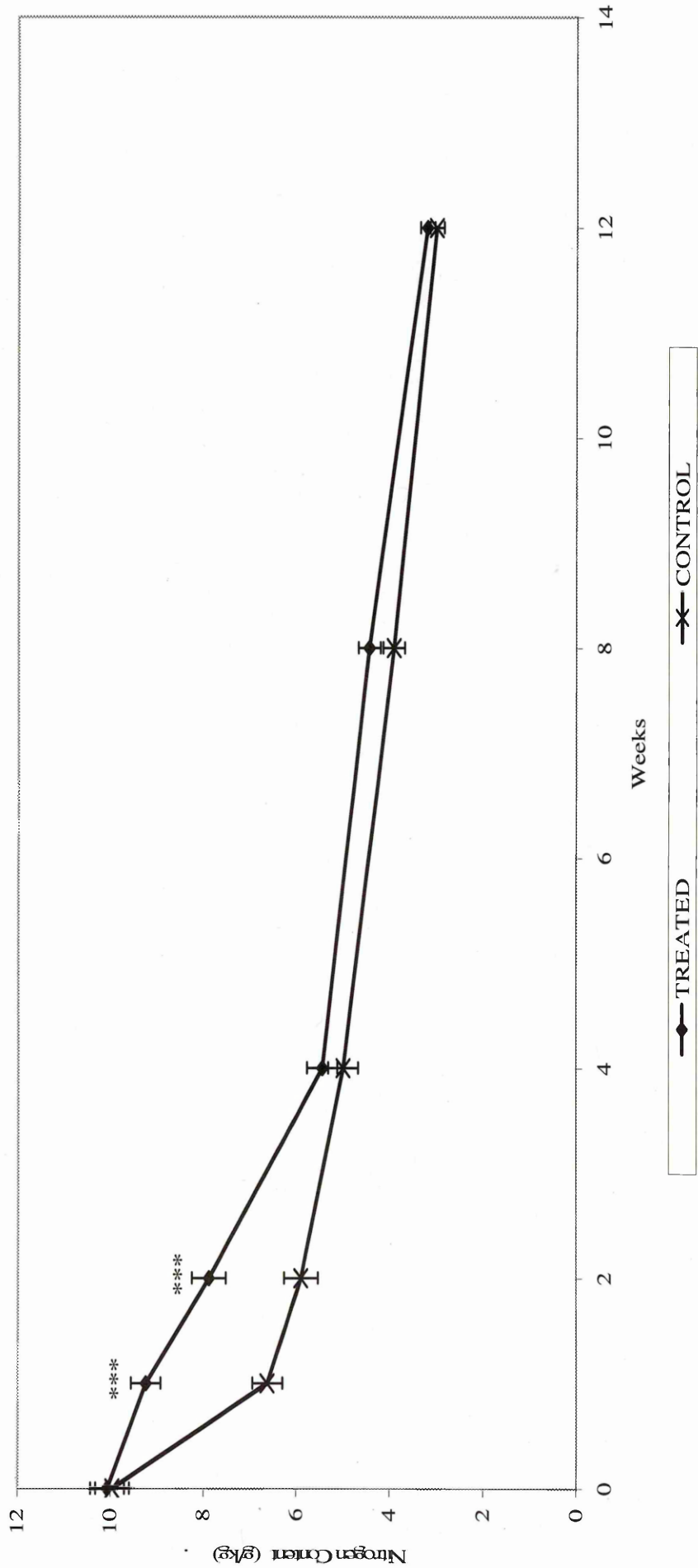
There was a slower increase in the early C:N ratio of the treated manures than in the control manure (P<0.001, Figure 8.6), but there was no difference in the final C:N ratio.

The quantity of dry matter and carbon present in the initial manure samples was affected by the sucrose amendment. The dry matter content of the amended manure remained significantly different from the control until week 2, whilst the carbon levels were significantly different until week 4. Both of these variables showed an interaction between the sucrose amendment and the length of storage time (P<0.001). The addition of sucrose to the manure produced a decrease in the pH level during the first week of storage (P<0.001 - Figure 8.7).

Table 8.8 The effect of sucrose addition on changes in the composition and characteristics of laying hen manure stored over 12 weeks.

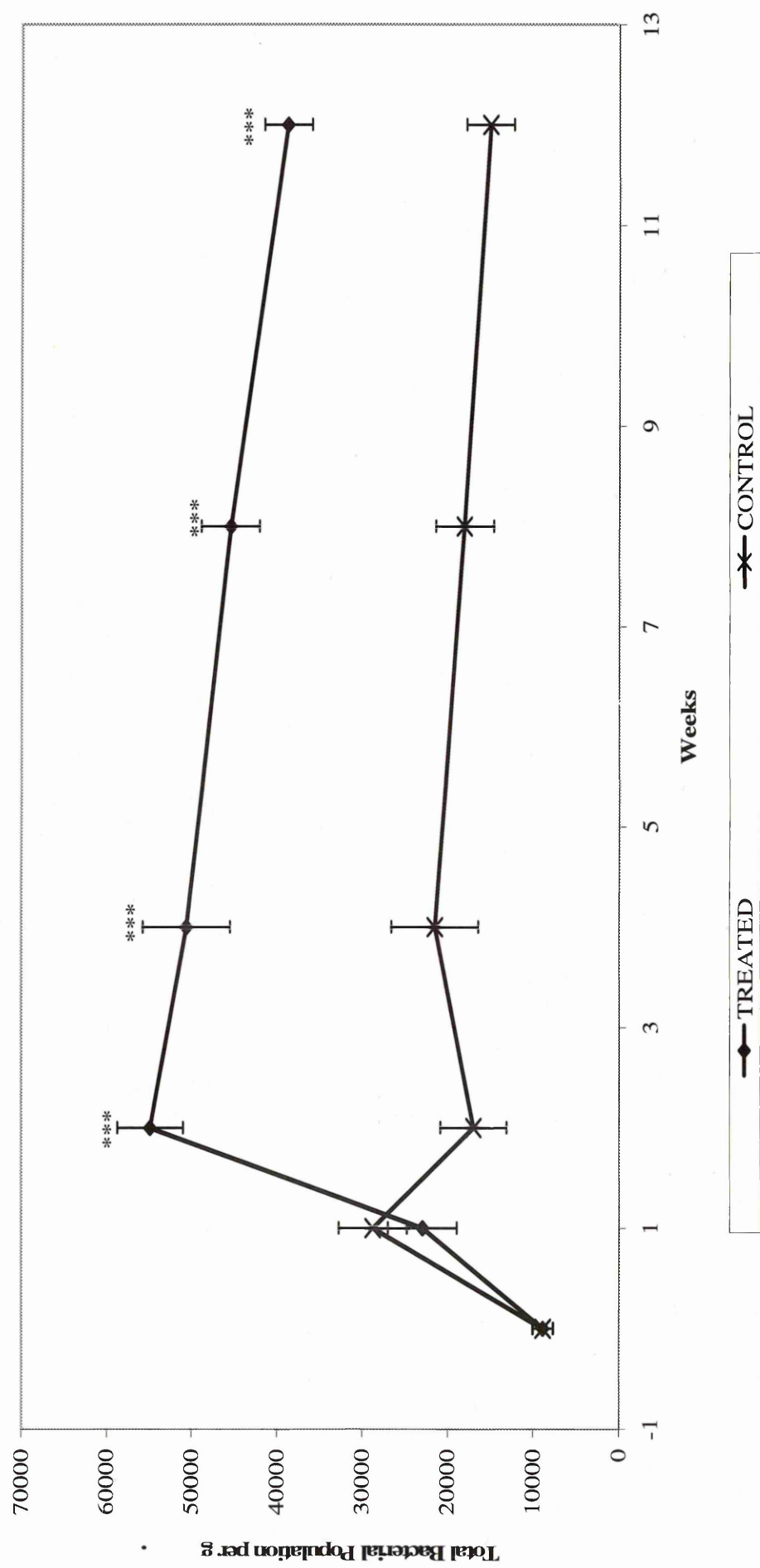
	Week	0	1	2	4	8	12	Time x Treatment Interaction
Dry Matter (g/Initial kg)	Treated	295.4	254.1	234.3	188.2	165.5	123.5	P<0.001
	Control	268.4	226.5	215.0	194.1	160.2	126.8	
	S.E.M.	2.72	3.81	5.51	5.92	7.32	4.14	
	Significance of Difference	P<0.001	P<0.001	P<0.05	P>0.05	P>0.05	P>0.05	
Water (g/Initial kg)	Treated	704.6	680.9	657.2	647.3	538.0	501.0	P>0.05
	Control	731.6	715.6	691.8	651.3	572.0	496.0	
	S.E.M.	2.72	4.35	7.46	10.69	18.60	17.20	
	Significance of Difference	P<0.001	P<0.001	P<0.01	P>0.05	P>0.05	P>0.05	
Nitrogen (g/Initial kg)	Treated	10.07	9.24	7.89	5.46	4.45	3.22	P<0.001
	Control	9.96	6.62	5.91	5.01	3.93	3.02	
	S.E.M.	0.365	0.319	0.364	0.325	0.231	0.154	
	Significance of Difference	P>0.05	P<0.001	P<0.001	P>0.05	P>0.05	P>0.05	
Carbon (g/Initial kg)	Treated	115.0	101.8	90.6	70.3	58.7	45.1	P<0.001
	Control	103.7	87.6	81.1	71.4	55.6	45.4	
	S.E.M.	1.44	1.24	1.79	2.11	2.40	1.52	
	Significance of Difference	P<0.001	P<0.001	P<0.001	P>0.05	P>0.05	P>0.05	
C:N	Treated	11.65	11.24	11.86	13.46	13.38	14.28	P<0.001
	Control	10.58	13.32	13.89	14.40	14.29	15.13	
	S.E.M.	0.399	0.298	0.376	0.457	0.331	0.314	
	Significance of Difference	P>0.05	P<0.001	P<0.001	P>0.05	P>0.05	P>0.05	
Sulphur (g/Initial kg)	Treated	0.52	0.48	0.46	0.35	0.34	0.31	P>0.05
	Control	0.51	0.44	0.42	0.35	0.34	0.32	
	S.E.M.	0.009	0.013	0.013	0.015	0.016	0.011	
	Significance of Difference	P>0.05	P>0.05	P<0.05	P>0.05	P>0.05	P>0.05	
pH	Treated	7.58	6.70	7.75	8.64	8.68	8.77	P<0.001
	Control	7.55	8.06	8.30	8.49	8.72	8.88	
	S.E.M.	0.043	0.123	0.152	0.065	0.029	0.052	
	Significance of Difference	P>0.05	P<0.001	P<0.05	P>0.05	P>0.05	P>0.05	
Electrical Conductivity (mS)	Treated	1.66	2.42	2.09	1.97	2.01	2.23	P>0.05
	Control	1.81	2.30	2.10	2.06	2.04	2.16	
	S.E.M.	0.018	0.063	0.051	0.037	0.036	0.055	
	Significance of Difference	P<0.001	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	
Bacteria (x 10 ⁶)	Treated	0.89	2.30	5.49	5.07	4.56	3.89	P<0.01
	Control	0.88	2.88	1.71	2.16	1.82	1.52	
	S.E.M.	0.12	0.40	0.39	0.51	0.34	0.28	
	Significance of Difference	P>0.05	P>0.05	P<0.001	P<0.001	P<0.001	P<0.001	
Water Activity	Treated	0.988	0.984	0.988	0.974	0.965	0.860	P<0.01
	Control	0.990	0.990	0.982	0.972	0.944	0.976	
	S.E.M.	0.0010	0.0026	0.0026	0.0040	0.0096	0.0204	
	Significance of Difference	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P<0.001	

Figure 8.4 The nitrogen content of laying hen manure stored over 12 weeks with and without the addition of sucrose at 35g/kg.



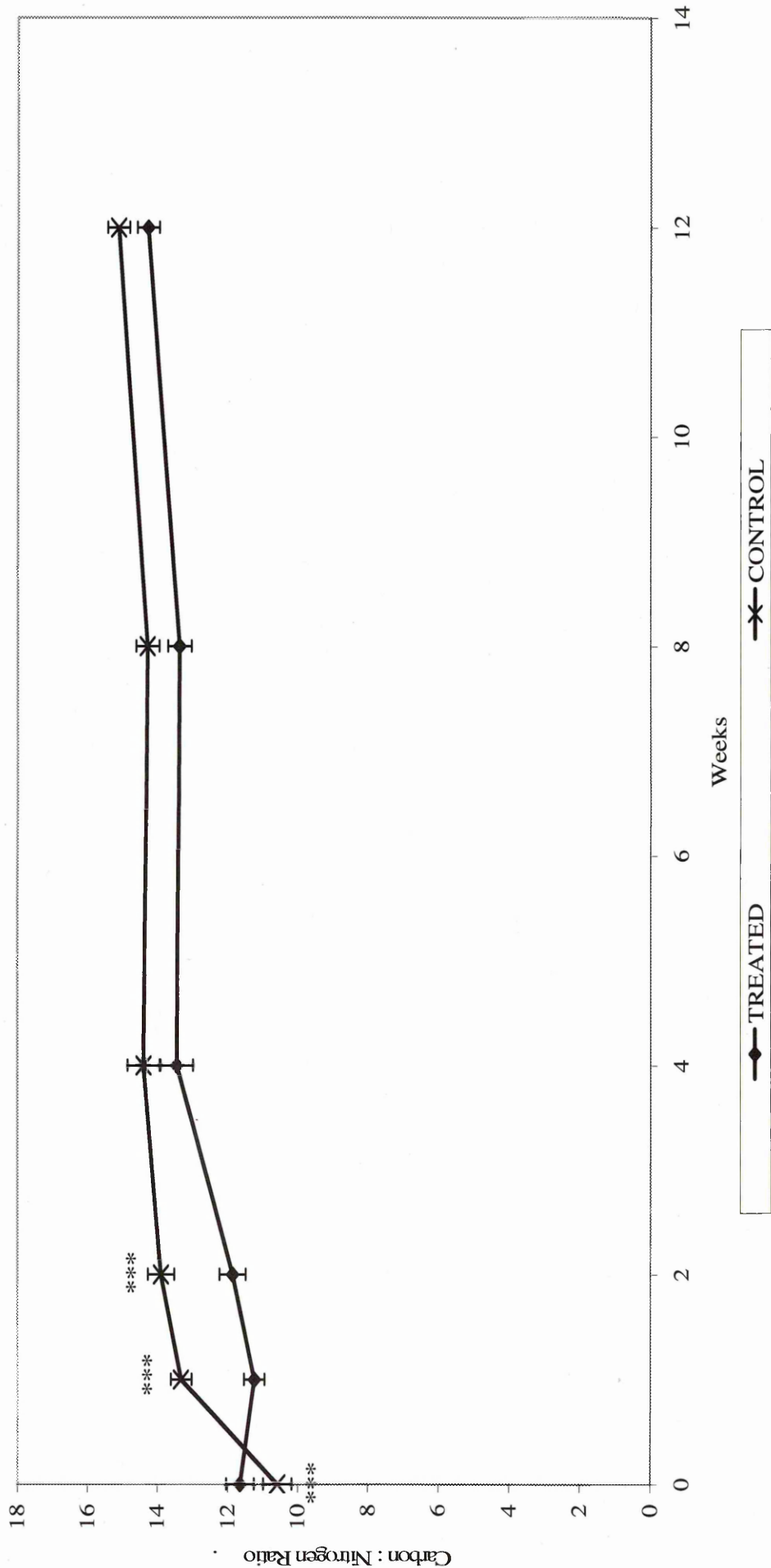
*** = $P < 0.001$

Figure 8.5 The bacterial population in laying hen manure stored over 12 weeks with and without the addition of sucrose at 35g/kg.



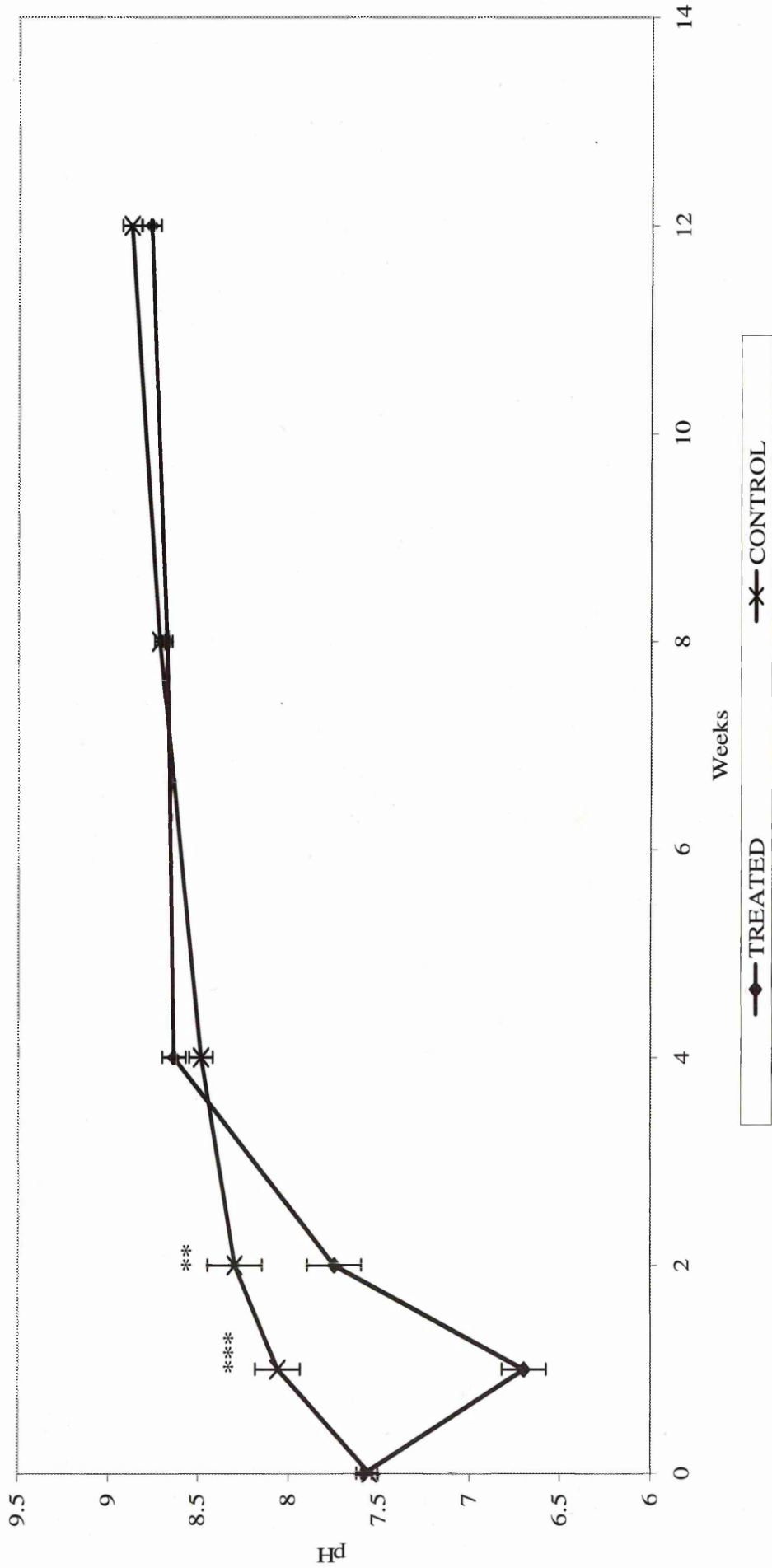
*** = P<0.001

Figure 8.6 The carbon to nitrogen ratio of laying hen manure stored over 12 weeks with and without the addition of sucrose at 35g/kg.



*** = P<0.001

Figure 8.7 The pH of laying hen manure stored over 12 weeks with and without the addition of sucrose at 35g/kg.



** = P<0.01 *** = P<0.001

By week 2, the pH had risen back to its original level and by week 4 it had risen to the same level as the untreated manure. The addition of sucrose had no effect on the water activity of the samples for the first 2 weeks. Following this period the water activity (which is a measure of the un-bound water in the sample) was reduced. The final water activity measurement at week 12 showed a significant drop in the water activity of the treated samples ($P < 0.001$).

8.4.4 Discussion.

The total nitrogen lost from the manure samples over the 12-week storage period was not affected by the addition of sucrose. Nitrogen losses over the 12-week storage period (70% of initial nitrogen lost) were similar to those reported by Williams *et al.* (1999) and Kirchmann & Witter (1989). But, there was an interaction between the addition of the sucrose and the length of storage. Nitrogen losses were significantly slower from sucrose amended manure until week 4. The addition of sucrose to the manure increased bacterial numbers after two weeks and there was an interaction between the amendment and the storage time, where the increase in bacterial numbers significantly increased in sucrose amended manure after week 2.

The addition of sucrose to the manure produced a decrease in the pH level during the first week of storage. This probably occurred as a result of the anaerobic decomposition of the sucrose producing acid compounds (Begon *et al.*, 1990). By week 2, the pH had risen back to its original level and by week 4 it had risen to the same level as the untreated manure. The pH fall measured in the treated manure may also be responsible for the nitrogen retention measured during the initial weeks of the experiment, though a degree of incorporation in the bacterial biomass may still have occurred as nitrogen was still retained until approximately week 4.

An increased C:N ratio indicates that more nitrogen is being lost than carbon (Kirchmann & Witter, 1989). Therefore, a slower increase in the early C:N ratio of the treated manures than in the control manure indicated a reduced level of nitrogen loss. Although, there was no difference in the final C:N ratio, there was an interaction between the amendment and the storage time showing that the slower increase in the C:N ratio was a significant treatment effect. The quantity of dry matter and carbon present in the initial manure samples was affected by the sucrose amendment. The dry matter content of the amended manure remained significantly different from the control until week 2, whilst the carbon levels were significantly different until week 4. Both of these variables showed an interaction between the sucrose amendment and the length of storage time. This increased level of carbon loss during these initial few weeks is indicative of an increased level of bacterial respiration (Golueke, 1992). The increased bacterial population measured in the treated manure may be linked with the nitrogen losses which occur more slowly during the weeks where the bacterial population has increased, indicating that nitrogenous compounds are utilised as energy sources in the absence of carbonaceous sources.

The initial drop in the electrical conductivity of the treated samples compared to the control probably occurred as a result of ions being released due to the increased microbial activity breaking down ions bound in the organic matter. As the decomposition of the un-treated control caught up so the level of the electrical conductivity stabilised between the two treatments. There was no interaction between the amendment and the length of storage time.

The addition of sucrose had no effect on the water activity of the samples for the first 2 weeks. Following this period the water activity (which is a measure of the un-bound water in the sample) was reduced. The final water activity measurement at week 12

showed a significant drop in the water activity of the treated samples ($P < 0.001$), this may account for the drop in bacterial numbers at this point.

8.4.5 Conclusions.

The addition of sucrose to fresh laying hen manure reduced the nitrogen loss for up to three weeks. Thereafter nitrogen was rapidly lost and there was no further benefit of the sucrose amendment. At the end of the 12-week storage period there were no differences between the treatments with respect to nitrogen content. The addition of a more resistant carbohydrate, such as straw, in conjunction with sucrose may provide a more lasting effect.

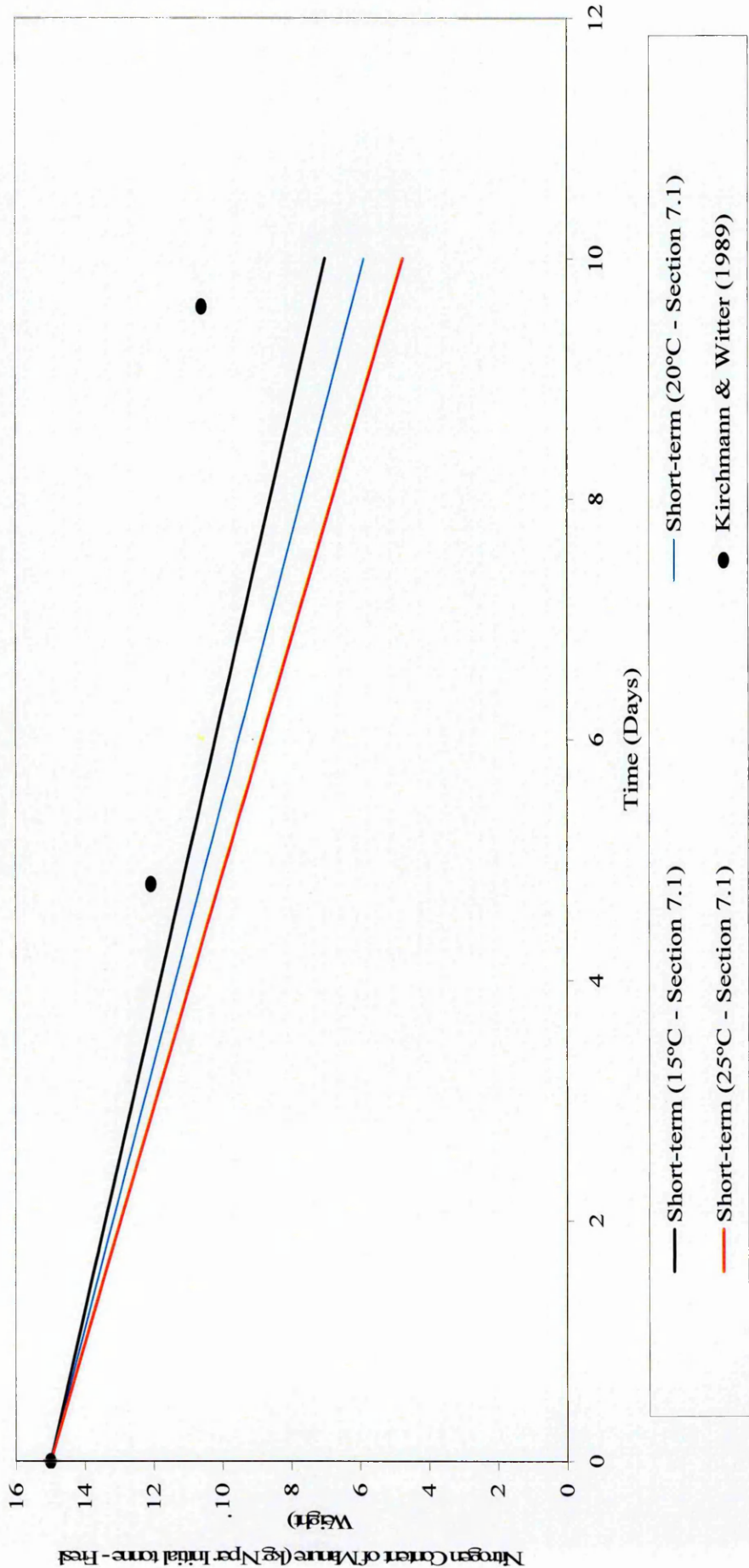
9. General Discussion.

9.1 The Effect of Ambient Temperature and Manure Moisture during the Storage of Caged Laying Hen Manure.

A main objective of this project was to examine, describe and quantify nitrogen loss from stored laying hen manure.

The overall pattern of nitrogen loss over time is unlikely to be linear as there would be a reduction in the rate of nitrogen losses, as the nitrogen content of the manure itself falls. The linear rates of loss determined from the experiments described in sections 7.1 and 7.2 represent only a portion of the whole process. The initial short-term losses are similar to those presented by Kirchmann & Witter (1989) (Figure 9.1). The linear nitrogen loss from manure during short-term (up to 10 days) storage probably occurred as a result of the continued water loss which facilitated nitrogenous losses. The reduction of the manure moisture content is important when attempting to minimise nitrogenous losses, but techniques that reduce the manure moisture content also result in increased ammonia release (Groot Koerkamp *et al.*, 1998). The evaporation rate of water can be increased by increasing temperature and air flow, and by decreasing the water vapour pressure. These factors also increase the rate of ammonia volatilisation from the manure. However, this increase in ammonia loss will not continue indefinitely as the removal of water from the manure will reach critical levels for microbial activity below which decomposition will slow, or even stop. The small sample sizes used in this experiment may also have facilitated nitrogenous and moisture losses. A small sample has a large surface area to volume ratio, and hence a larger emitting surface.

Figure 9.1 The nitrogen loss from laying hen manure during short-term storage: The main treatment effects (Experiment 7.1 - lines) compared to other published data.



This may have been a factor in the linear losses produced during the short term experiments which differ from other published work e.g. Burnett & Dondero (1969) who found exponential losses.

The increased rate of nitrogen loss (which remains linear) during short-term storage results from the increase in temperature which raises microbial activity. There was a positive linear effect of temperature on the rate of water loss from the manure. As temperature increases the capacity of the atmosphere to hold water, water may be removed from the surface of the manure substrate more rapidly hence, increasing, evaporation rate. The pH level was significantly increased at higher ambient storage temperatures, indicating an accumulation of ammonia (Fowler *et al.*, 1996). This increase in the pH level also resulted in an increased nitrogenous loss from the manure as the loss of ammonia can only occur whilst it is present in the manure solution in this un-ionised form (Elliott & Collins, 1982).

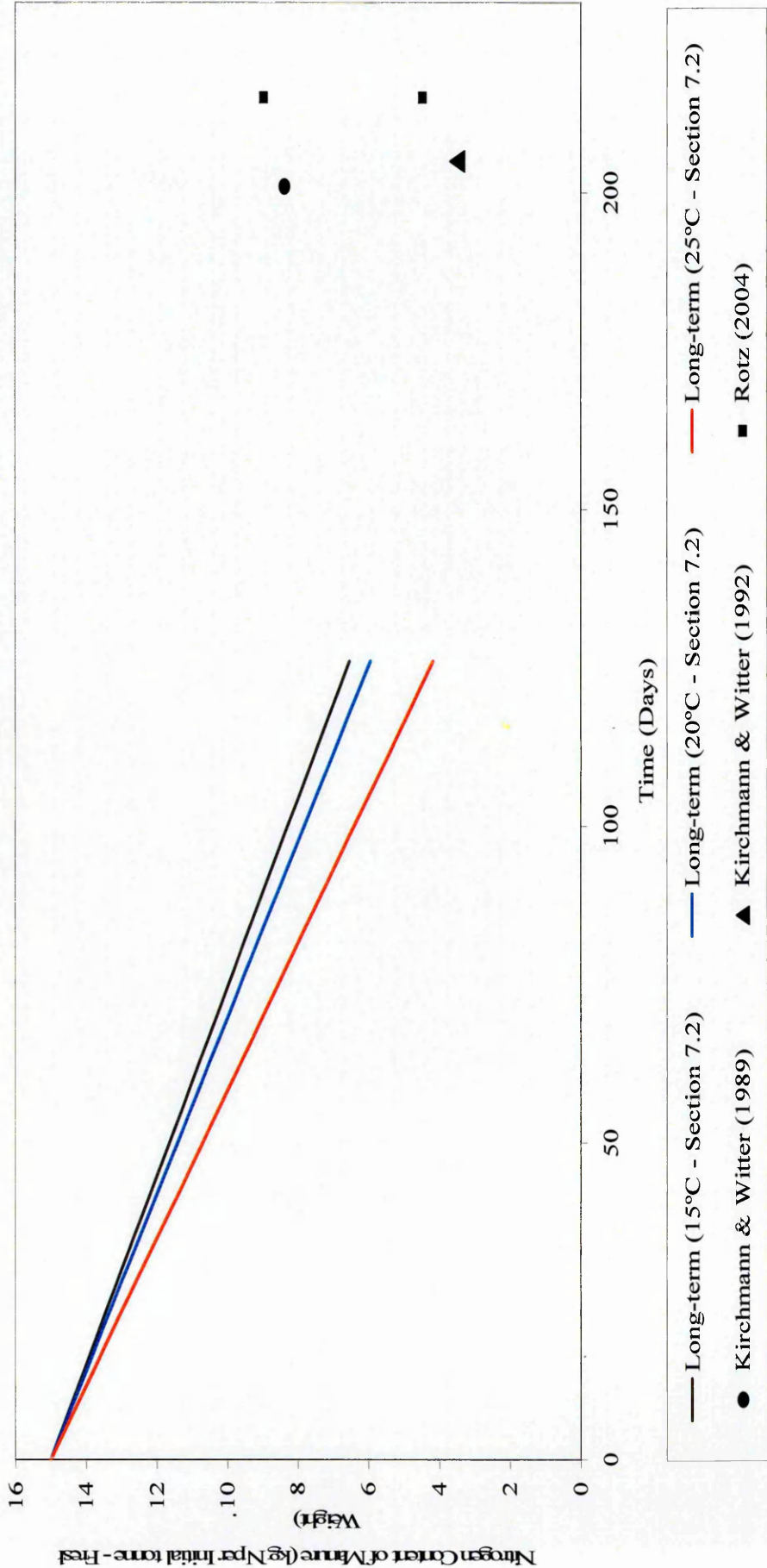
The effect of initial moisture content was also examined during the short-term storage of laying hen manure (Section 7.1). All of the manure moisture contents were initially above the optimum range for microbial activity (40-60%) suggested by Elliott & Collins (1983 -Figure 3.5) for poultry litter. However, as there was a significant non-linear effect of initial manure moisture content on the rate of nitrogen loss within this range, it may be suggested that the optimum range for microbial activity differs for poultry manure, as shown by the interaction between ambient storage temperature and initial egesta moisture content (Section 7.1). These data showed a large increase in nitrogen loss for the intermediate moisture treatment (752 g/kg) indicating a possible optimum in the region of 75% moisture.

The combinations of the factors studied are indicative of the complex, inter-related nature of biological decomposition. While the data only supported an interaction between storage temperature and initial moisture content with respect to nitrogen loss, it is not un-reasonable to suggest that microbial activity would also be significantly affected by both parameters.

Removal of manure from the caged area as soon as possible following excretion is recommended as storage temperatures within the cage area promote the formation and release of ammonia. This would not only benefit the welfare of the work force and stock, but reduce nitrogen emissions from the cage area. Minimisation of manure moisture would also be beneficial for ammonia reduction both within the housing system and during manure storage. This may be achieved by optimising dietary minerals (Smith *et al.*, 2000a) and good drinker management (Phillips & Chambers, 2002).

In contrast to short-term storage, the long term-storage of manure normally occurs in large heaps. In order to examine nitrogen losses from the long-term storage of manure one tonne of caged laying hen manure produced over a 48 hour period was used for each experimental unit. As a result of this large sample size, the surface area to volume ratio was smaller. The lower surface area to volume ratio resulted in slower rates of nitrogen loss (Section 7.2) compared to in smaller samples (Section 7.1 - Figure 9.1), but for a longer duration (Figure 9.2). The non-linear nature of the effect of temperature on the rate of nitrogen loss during the long-term storage of caged laying hen manure indicates its importance during long-term manure storage. Storage temperatures below 20°C have very little influence on nitrogen losses therefore deep-pit manure stores would only produce additional nitrogenous losses compared to external storage areas, if the temperature in the store exceeded this level.

Figure 9.2 Estimates of the nitrogen loss from laying hen manure during long-term storage and a projected pattern of loss over a year's storage: Experiment 7.2 and relevant published data.



Manure stored outside the housing system would be subject to ambient environmental temperatures which could not be controlled and although air temperatures do not normally exceed 20°C in the U.K. for long periods of time there are periods particularly in the summer months where temperatures do exceed this level. Protection of the manure from inclement weather is also required for manure stored outside the housing system. Although chamber ventilation rate and the effect of air flow over the manure heap were not variables studied during this project, their effects may become more prominent when manure is stored away from the housing system. As the mass transfer coefficient of ammonia is increased with increasing air flow over the manure surface (Section 4.1.2), nitrogenous losses would be increased when manure is exposed to an increased level of air flow. This was highlighted during the study (ADAS, 2002) of stilt housing systems in which the authors noted that the losses may have been underestimated due to the wind flow over the exposed manure surfaces. Therefore, protection of the manure store from wind and rainfall is likely to be beneficial when attempting to reduce ammonia emission. Whilst temperature control is important, it is the combination of environmental factors that produce the final ammonia emission rates.

The financial cost of the emission of ammonia from laying hen housing can not be specifically calculated, as factors such as costs to plant and animal species and the wider environment can not easily be determined in monetary terms. The following calculations aim to put the loss of nitrogen from laying hen manure into a monetary context by comparison with the replacement cost of the fertiliser nitrogen lost to the environment.

The financial cost of nitrogen loss from stored manure can be crudely estimated using the cost of purchasing nitrogen fertiliser to replace the volatilised nitrogen. The cost of

replacing the nitrogen lost during short-term and long-term storage is presented in Table 9.1 and Table 9.2, respectively. These calculations clearly illustrate the rise in cost to replace the lost nitrogen from stored nitrogen as temperature increases. To conserve fertiliser nitrogen it is essential that fresh manure be removed from the warm caged area as soon as possible after production and stored in large heaps away from the stocked area.

Table 9.1 The estimated cost of replacing nitrogen lost during short-term manure storage.

	Ambient Storage Temperature	Rate of Nitrogen Loss	Cost of Nitrogen Lost ^{*1}	Cost per 1000 Laying Hens ^{*2}
	°C	kg t ⁻¹ week ⁻¹	£ t ⁻¹ week ⁻¹	£ week ⁻¹
First Week of Storage	15	5.582	2.009	82.38
	20	6.369	2.293	94.01
	25	7.157	2.576	105.63

^{*1} Average price of nitrogen fertiliser = £360 / tonne (Nix, 2004).

^{*2} Data based on 41 tonnes of undiluted manure produced from 1000 laying hens per year (MAFF, 2000).

Table 9.2 The estimated cost of replacing nitrogen lost during long-term manure storage (Six month storage period).

	Ambient Storage Temperature	Rate of Nitrogen Loss	Cost of Nitrogen Lost ^{*1}	Cost per 1000 Laying Hens ^{*2}	Cost per Six Months Storage
	°C	kg t ⁻¹ week ⁻¹	£ t ⁻¹ week ⁻¹	£ week ⁻¹	£
Six Months of Storage	15	0.469	0.169	6.93	180.06
	20	0.502	0.181	7.41	192.58
	25	0.601	0.216	8.86	230.47

*1 Average price of nitrogen fertiliser = £360 / tonne (Nix, 2004)..

*2 Data based on 41 tonnes of undiluted manure produced from 1000 laying hens per year (MAFF, 2000).

The effect of manure moisture content was also examined during the short-term storage of laying hen manure (Section 7.1). These data were used to estimate the financial cost of nitrogen loss during early manure storage for a range of moisture contents and temperatures, using the cost of purchasing fertiliser nitrogen to replace the volatilised nitrogen (Table 9.3). The calculations again illustrate the cost impact of allowing higher moisture content in stored manure. Though at the highest moisture content studied (796 g/kg), costs were lower due to the reduced nitrogen losses produced by this treatment. This effect was consistent with other published data (Stentiford, 1996; Miller, 1989; Schefferle, 1965a) who also found a reduction in nitrogenous losses at high moisture contents, which they attributed to the presence of anaerobic activity.

However, the lowest cost and hence the lowest nitrogen losses were achieved from the lowest moisture content tested (722 g/kg) and the lowest storage temperature (15°C).

Table 9.3 The estimated cost to replace nitrogen lost during the early storage of laying hen manure of differing initial moisture content at a range of temperatures.

Initial Moisture Content	Ambient Storage Temperature	Rate of Nitrogen Loss	Cost of Nitrogen Lost	Cost per 1000 Laying Hens ^{*2}
g kg ⁻¹	°C	kg t ⁻¹ week ⁻¹	£ t ⁻¹ week ⁻¹	£ week ⁻¹
722	15	5.582	2.010	82.39
	20	6.370	2.293	94.02
	25	7.157	2.577	105.64
752	15	5.985	2.155	88.34
	20	8.400	3.024	123.98
	25	10.815	3.893	159.63
796	15	5.916	2.130	87.32
	20	6.905	2.486	101.92
	25	7.895	2.842	116.53

*1 Average price of nitrogen fertiliser = £360 / tonne (Nix, 2004)..

*2 Data based on 41 tonnes of undiluted excreta produced from 1000 laying hens per year (MAFF, 2000).

Lowering the moisture content of manure is of practical importance as there is the potential to reduce nitrogen losses significantly by ensuring that there are no dietary mineral excesses (Smith *et al.*, 2000a & b) and that good water management practices are in place (Phillips & Chambers, 2002). Methods of control of manure moisture content are likely to be more effective than attempting to dry the manure using temperature or ventilation levels once it is produced. However, much greater reductions in moisture content than could be practically achieved through diet and water management are likely to be required to completely prevent nitrogen loss. This restriction of water may halt the decomposition of the manure temporarily but this could introduce other environmental problems elsewhere when the manure is applied to the land resulting in the release of ammonia when decomposition recommences. Microbial manure decomposition results in a reduction of mass through respiration of carbonaceous and nitrogenous compounds. This results in limited self-heating which can result in a reduction of the heat labile pathogenic bacteria. This process stabilises the manure ensuring its application to land is not detrimental to the soil-crop interface. If dry, un-decomposed manure is added to land, it would adsorb moisture from the soil and biological processes would be resumed, which may result in soil ammonia concentrations that are toxic to the root system of the crop.

The moisture content of laying hen manure must be minimised by ensuring there are no dietary mineral excesses and by good water management practices. The drying of manure using the normal temperature and ventilation levels underneath the cages is not recommended as it results in large quantities of ammonia being produced and emitted. Manure should be removed from the cage area as soon as possible.

9.2 The Addition of Carbonaceous Amendments to Caged Laying Hen Manure.

The incorporation of ammonia into new N-containing organic compounds by microbial immobilisation requires C:N ratios of at least 30 which are not normally present in caged laying hen manure. The addition of a carbon source to laying hen manure could instigate microbial immobilisation of the nitrogen present. The availability of the carbon compounds needs to coincide with the production of the ammonia so that it can be incorporated into the microbial population. Potentially this may result in large quantities of nitrogen being retained in the microbial biomass, preventing its gaseous loss. The addition of a natural carbon source such as sucrose, straw or starch to the laying hen manure would not prevent the manure from being used as a fertiliser.

The decomposition of laying hen manure prior to its application to agricultural land is important to prevent the detrimental effects of nitrogen toxicity to the soil-crop system. Composting is one of the most effective methods of producing a stable, beneficial soil conditioner and fertiliser. The composting of laying hen manure is problematic as it has a very low C:N ratio, which results in large nitrogen losses, and is generally too wet to facilitate aerobic decomposition. Therefore, to utilise laying hen manure to its full potential a carbonaceous amendment added to the manure will aid decomposition. Composting is a biological process (Section 3.7) that incorporates the available carbon and nitrogen into the microbial biomass. If an appropriate balance can be achieved by the addition of available carbon, the available nitrogen produced within the manure can be incorporated into the biomass instead of being emitted to the atmosphere. This would be of enormous benefit, as the nitrogen would remain “locked” within the microbial biomass.

Laying hen manure contains very little available carbohydrate as a result of the highly digestible diets that are fed. If nitrogen is to be retained by incorporation into the biomass, an additional source of carbohydrate must be added. Cage systems with mechanical belt-scraped manure removal systems offer an opportunity to add an amendment as part of this process. During the first week of storage laying hen manure is especially vulnerable to nitrogenous losses, as it is often still stored within the cage area where temperature, egesta moisture content and air movement produce large nitrogen losses (Section 7.1). A carbohydrate source would need to be available to the microbial population at this stage of storage.

The initial manure compositions used in sections 8.1, 8.2 and 8.3 were similar to those found by other authors (Nicholson *et al.*, 1996). The mean losses of nitrogen in these three sections were 520 g/kg, 300 g/kg and 240 g/kg respectively and were in the same range as the losses measured by Williams *et al.* (1999) and Rotz (2004). The nitrogen loss at week 1 in the experiment presented in section 8.4 was comparable to the nitrogen lost for the same amendment level in section 8.3. The greater nitrogen losses in experiment 8.1 than in experiments 8.2 and 8.3 were probably a consequence of the shorter storage times prior to the start of the experimental storage periods and the smaller sample sizes that were stored. The smaller sample sizes used in experiment 8.1 had higher surface area to volume ratios, which may have allowed a greater volatilisation rate. The slight differences in initial dry matters between the three experiments probably had little effect on the overall nitrogen loss, and the water activities of all the initial samples were in excess of 0.9 which is well above the level needed for bacterial growth (Griffin, 1981). The initial bacteria levels measured 1×10^4 - 10×10^4 were much lower than that reported by Schefferle (1965a), 88×10^7 and Halbrook *et al.* (1951), 1×10^6 - 100×10^6 . As these authors examined fresh manure,

whereas the manure examined in this project was freeze-dried, so some proportionate loss of viable microbial numbers was expected. Chang *et al.* (1974) cultured microorganisms from dehydrated manure and produced similar results (6×10^3 - 3×10^6) to that produced in this project.

A quantitative evaluation of the ability of a range of carbohydrates (glucose, sucrose, starch and straw) to reduce nitrogen loss was discussed in section 8.1. The addition of glucose to laying hen manure produced over a 48 hour period significantly increased the nitrogen lost over the next 7 days compared to the control. This was presumably because it provided an immediate energy source to the microbial population, which did not coincide with the release of ammonia. This doubled the microbial population that resulted in the release of the greater amount of nitrogen. The straw treatment had no effect on the nitrogen loss or the microbial population during this period. It is likely that the short storage period did not allow sufficient time for the breakdown of the straw making it unavailable to the microbial population. The sucrose and starch amendments were of more interest as they both tended to show a reduced level of nitrogen loss. The addition of sucrose enabled the incorporation of nitrogen into the biomass, as it significantly increased the bacterial population to almost three times that of the control (Section 8.2), whilst still showing the potential for reduced nitrogen loss. There was also a significant increase in the amount of carbon lost from the sucrose amended manure. This is important, as it is evidence of enhanced microbial respiration and implies nitrogen is being incorporated into the biomass (microbial immobilisation – section 3.5).

These data suggest that carbohydrates with a carbon availability similar to that of sucrose, may make suitable amendments for reducing nitrogen loss from laying hen manure during its early storage and warrant further investigation.

The evaluation of carbohydrates with similar microbial availability to sucrose was conducted (Section 8.2). Both sucrose and maltose produced significant reductions in nitrogen loss, and produced increased bacterial populations. The starch and α -amylase treatment had no significant effect on nitrogen losses, unlike the result found in the earlier experiment (Section 8.1). This suggests that if starch were to be used as an amendment it may be more effective without the aqueous enzyme, as the aqueous portion increased the moisture content, which is known to increase nitrogen losses (Elliott & Collins, 1982).

These data confirm the effectiveness of disaccharides at providing an available carbon source for the microbial population, allowing the microbial population to incorporate the nitrogen compounds present into their biomass during the early storage of caged laying hen manure.

The quantity of available carbon provided in the amendment was also thought to have an affect on nitrogen retention. But, increasing the quantity of sucrose added to the manure did not increase the amount of nitrogen retained in a linear manner. The data presented in section 8.3 revealed a significant non-linear effect of increasing levels of sucrose inclusion which did not coincide with the peak level of the microbial population. This may be related to changes in the pH of the decomposing manure. As the quantity of the sucrose inclusion increased, the size of the pH increase was reduced. At the optimum inclusion rate for nitrogen retention (Section 8.3) and above, the manure pH was reduced below the initial level. This presumably occurred as a result of acid formation during anaerobic fermentation (Begon et al., 1990) or during carbon dioxide production as a result of microbial respiration (Witter & Kirchmann, 1989b). In either case the pH levels were not conducive for ammonia production and release. The reduced nitrogen loss and the lack of increase of the bacterial population may also be

explained by the low pH, as nitrogen is not lost as ammonia from acidic conditions and the microbial population may not have performed as efficiently at the more acidic pH. The pH shift would also have caused an increase in the proportion of ammonia produced by the bacteria to be bound in a non-volatile form (ammonium ion). The acidifying effects of carbohydrate additions to manure were also shown by Clemens *et al.* (2002) who also showed a non-linear relationship between the level of acidification (and hence nitrogen losses) and the concentration of sucrose amendment.

As discussed in section 9.1, the financial cost of nitrogen loss from stored manure can be estimated using the cost of purchasing fertiliser nitrogen to replace the volatilised nitrogen. In the case of nitrogen retention, savings in fertiliser nitrogen can be achieved. The potential savings produced by retaining nitrogen in the manure compared to the rate at which the amendment is applied is presented in Table 9.4. Due to the non-linear nature of the effect, there was no increase in savings above an amendment level of 20 to 35 g/kg. However, as sucrose is an expensive carbohydrate the cost of the amendment is greater than the value of the nitrogen saved (Table 9.4). If penalties are introduced for environmental pollution, this method of nitrogen retention may become economically feasible.

Table 9.4 Calculation of the replacement value of nitrogen retained by a sucrose amendment at increasing levels and overall savings.

Level of Sucrose Addition	Nitrogen Lost	Nitrogen Retained compared to control	Value of Nitrogen Retained* ¹	Value of Nitrogen Retained per 1000 Laying Hens* ²	Value of Nitrogen Retained over 3 weeks per 1000 Laying Hens* ^{2*4}	Cost of Sucrose Application per 1000 Laying Hens* ^{2*3}	Cost to Producer Per 1000 Laying Hens
kg t ⁻¹	kg t ⁻¹ week ⁻¹	kg t ⁻¹ week ⁻¹	£ t ⁻¹ week ⁻¹	£ week ⁻¹	£	£	£
0	5.06	0	0	0	0	0	0
5	3.83	1.23	0.44	18.16	54.48	92.66	38.18
10	3.29	1.77	0.64	26.12	78.36	185.32	106.96
20	2.30	2.76	0.99	40.75	122.25	370.64	248.39
35	0.99	4.07	1.47	60.07	180.21	648.62	468.41
50	1.50	3.56	1.28	52.52	157.56	926.60	769.04

*1 Average price of nitrogen fertiliser = £360 / tonne (Nix, 2004).

*2 41 tonnes of undiluted excreta produced from 1000 laying hens per year (MAFF, 2000).

*3 Price per kg of sucrose £0.45 (Courtesy of Napier Brown).

*4 3 week effectiveness of amendment estimated from section 8.4.

The duration of the nitrogen retaining effects of sucrose amendments were investigated.

Over an extended storage period the optimum inclusion rate established for sucrose (Section 8.3) had no significant effect on nitrogenous losses. Closer examination revealed the sucrose amendment significantly retarded nitrogen losses for the first two

weeks. There was a similar affect on the bacterial numbers and pH level. The amended manure had a decreased pH during this period of storage which was restored to its initial value after the second week of storage. Once the pH was restored to this level, the bacterial population was significantly increased until the end of the experiment. This lag period would appear to be directly related to the pH level. The pH level has been shown to be the most important parameter affecting ammonia loss (Elliott & Collins, 1982). It is possible that an additional effect of adding available carbohydrates to decomposing caged laying hen manure is the reduction of the pH level. Whether this is as a result of microbial respiration in aerobic decomposition (Witter & Kirchmann, 1989b) or as a result of acid formation during anaerobic decomposition (Begon et al., 1990) would be a subject of further study.

The inclusion of sucrose as an amendment to provide an available carbon source over the entire duration of the storage period is not an economically viable or effective option, the effects of providing the available carbon to the microbial population in the manure are of benefit in the long-term as: the pH level in the manure is reduced and the microbial population is increased, promoting nitrogen retention.

These factors remain worthy of further study and further investigations would be best served establishing the effects of the carbohydrate amendments on the pH level; establishing the rate of uric acid breakdown; determining the form of nitrogen present following the breakdown of uric acid and how these compounds are utilised by the microbial population. However, the first task would be to determine if a more economical carbohydrate source exists.

The use of carbohydrates to reduce nitrogen losses from stored manure shows considerable promise, but the use of sucrose alone is both uneconomic and only provides for nitrogen retention in the early stages of decomposition. A possible

alternative to overcome both of these difficulties could be the development of a mixed carbohydrate amendment with differing carbon availabilities. Sucrose could provide an available carbon source during the first two weeks, aided initially by the pH decrease and then by supplying available carbon to the microbial population allowing it to incorporate nitrogen into its biomass. Other components of this mixed amendment, such as straw or wood shavings could supply additional available carbon to the microbial population, as the carbonaceous substrates were decomposed. Another potential method of reducing nitrogen emissions using carbonaceous amendments could be the use of sugar beet pulp. This may provide available sucrose and more resistant cellulose for a cheaper, perhaps more long-term solution than sucrose alone. The use of non-aqueous enzymes (e.g. cellulase) could also be investigated in conjunction with sugar beet pulp. Green wastes and organic household wastes could also be investigated. The development of mixed amendments of this type could be the subject of a further study.

10. Conclusions and Recommendations.

- Ammonia is produced from the microbial decomposition of nitrogenous components in manure. A range of factors (temperature, moisture content, pH, oxygen availability) can alter microbial growth, as well as parameters such as nutrient availability (e.g. C:N ratio). Potentially the most effective methods of controlling nitrogenous losses from laying hen housing systems are the control of ambient temperature and manure moisture levels.
- There was a linear effect of increasing temperature ($P < 0.01$) on the rate of nitrogen loss from caged laying hen manure during the first 10-days of storage. A 1°C increase in ambient storage temperature gave an 0.16 kg increase in nitrogen loss per tonne of stored manure per week. Therefore, reducing the temperature at which the manure is stored could substantially reduce the environmental nitrogen loss. The prompt removal of manure from the stocked area is recommended.
- The initial manure moisture content had a non-linear effect ($P < 0.001$) on nitrogenous loss during short-term storage (10 days). Increasing the initial manure moisture by 30 g/kg from 722 g/kg increased the rate of nitrogen loss by 23% (at 15.3°C). Minimising excess moisture is imperative and can be achieved by preventing excess dietary minerals and good drinker management.

- When ambient temperature exceeded 20°C during long-term manure storage, the rate of nitrogen loss was approximately three times greater than the rate of nitrogen loss when ambient storage temperature was below 20°C. Differences in the ambient temperature of manure storage systems below 20°C are not environmentally important when considering gaseous nitrogenous losses. The storage of manure at temperatures exceeding 20°C should be minimised.
- The addition of sucrose or maltose to caged laying hen manure gave a 40% reduction in nitrogen loss ($P<0.01$) and a 170% increase in bacterial numbers ($P<0.01$) during short-term storage. Also, increasing the concentration of the sucrose amendment produced a non-linear reduction in nitrogen loss ($P<0.01$), with an optimum inclusion rate in the region of 20 - 35 g/kg of sucrose during short-term storage. Sucrose or maltose may be added to fresh caged laying hen manure to reduce the loss of nitrogen during the initial weeks of storage.
- The addition of sucrose (35g/kg) to caged laying hen manure during long-term storage (12-weeks) had no significant effect on the overall loss of nitrogen, with approximately 70% of the initial nitrogen present being lost. However, there was a significant reduction in nitrogen loss ($P<0.001$) until week 4 in amended manure. Indicating the amendment was effective for up to 4 weeks. Therefore if sucrose was intended as a long term method of reducing nitrogenous losses, reapplications would be required.
- Further work is recommended to determine the effectiveness of sucrose as part of a mixed carbonaceous manure amendment to reduce nitrogenous losses during long-term storage of caged laying hen manure.

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Appendix 1

pH and Electrical Conductivity measurement.

The pH was determined by adding 50ml of distilled water to 1g of sample. The resulting suspension was mixed for one hour and the pH determined using an electronic pH probe and meter. The pH was determined from an un-dried sample for experiments 7.1, 7.2 and 8.1 and on a ground, freeze dried sample for experiments 8.2, 8.3 and 8.4.

Electrical conductivity was measured using an electronic conductivity meter from the same solution as used for pH determination.

Appendix 2

Determination of microbial population by reference to ATP content.

The microbial biomass was calculated from the microbial Adenoside-Triphosphate (ATP) content determined by a bioluminescence technique. Utilising a Luminoskan TL Plus and Quantitative ATP Monitoring kit, with an ATP Releasing Agent (ThermoLabsystems, Vantaa, Finland). The microbial ATP content was used to calculate the total biomass assuming 1×10^{-15} g microbial ATP corresponds to a single bacterial cell (Lundin, 1999).

Measurements were made from freeze dried samples (1g) reconstituted with 9ml of maximum recovery diluent (MRD, Oxoid, CM0733). MRD is designed to recover microbial cells from a dehydrated state and hold the population stable for 2 hours.

The samples were then incubated for 1 hour at 32°C using a shaking waterbath, allowed to stand for 15 minutes and then measured within the 2 hour stable period. The luminescence of 50µl of the sample solution, 50µl of ATP releasing agent and 50µl of ATP monitoring reagent was measured over 10 seconds (following a 2 second lag period).